ACTA ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 18 **March 1989** **Number 1**

Akadémiai Kiadó
Budapest

ISSN 0139—3006

CODEN ACALDI

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

Distributor:

KULTURA, Hungarian Foreign Trading Company
P.O. Box 149, H-1389 Budapest 62, Hungary

Publication programme, 1989: Volume 18 (4 issues).
Subscription prices per volume: US $ 130,00 plus 15% postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 18

1989

AKADÉMIAI KIADÓ
BUDAPEST

Acta Alimentaria, Vol. 18 (1), pp. 3–11 (1989)

ROLE OF DIETARY OILS IN THE REGENERATION OF EXPERIMENTAL LIPID METABOLISM DISTURBANCE

Á. J. N. ZSINKA[a] V. FÖLDES,[b] A. GERE,[b] J. PERÉDI[c] and T. THURÓCZY-THIERMAYER[d]

[a] Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary
[b] National Institute of Food Hygiene and Nutrition, H-1097 Budapest, Gyáli út 3/a. Hungary
[c] Vegetable Oil and Detergent Research Institute, H-1106 Budapest, Maglódi út 6. Hungary
[d] EGIS Pharmaceuticals, H-1106 Budapest, Keresztúri út 30–38. Hungary

Received: 1 April 1987; revision received: 28 March 1988; accepted: 29 March 1988)

Sexually mature male Wistar rats were fed isoenergetically for 6 weeks with a synthetic lipogenic diet containing 20 percent sunflower-oil or an oil mixture consisting of 33 percent sunflower-oil and 67 percent MCT C_8C_{10}-rich oil, to induce a fat metabolism disturbance. Recording the changes in certain lipid indices of the serum and liver, rats were fed for further 3 weeks with a normal synthetic diet containing 5 percent of the same oils. Lipid indices of serum and liver and the fatty acid composition of the liver were again determined at the end of this period. It has been found that

— the high lipid values of the serum and liver of rats, induced by the lipogenic diet, after a 3 weeks postfeeding with 5 percent fats in a normal synthetic diet showed the fat metabolism disturbance to become normalized;
— in the period of regeneration the high lipid level in the serum came closer to the normal level upon consumption of the fat mixture than on sunflower-oil alone;
— the high liver lipid values diminished after consumption of a normal synthetic diet with either of the fats;
the total cholesterol content of the liver however was significantly lower when the rats were fed the fat mixture in the normal diet than with sunflower-oil;
— the fatty acid composition of the liver approximated the control values upon consumption of both fats. As an effect of the fat mixture the total fatty acid content came near to the control value (P/S = 0.96), while upon consumption of sunflower-oil P/S = 1.7;
— by the end of the regeneration period the high $C_{18:2}/C_{20:4}$ quotient formed upon the consumption of the lipogenic diet diminished to a lower value (1.7) as an effect of the fat mixture, rather than upon sunflower-oil consumption (4.0).

Keywords: fat-metabolism disturbance, regeneration, C_8 C_{10} MCT/medium chain triglycerides/-rich fat mixture, serum and liver lipids

In our former study (J. N. ZSINKA et al., 1988a) rats were fed a synthetic lipogenic diet containing (among others) 1% cholesterol and 20% of either sunflower-oil or a mixture of sunflower-oil and oil rich in medium chain (C_8C_{10}) triglycerides (MCT). As an effect of both diets with very different P/S values, a substantial increase of the lipid indices of serum and liver was observed compared to the control values. However, the effects of the two oils differed: sunflower-oil itself caused significantly lower serum and higher liver lipid content, while the MCT-rich oil and sunflower-oil mixture gave higher serum and lower liver lipid values, as a consequence of MCT-oil, similarly to

 Akadémiai Kiadó, Budapest

data of experimental MCT-feeding by STEWART (1978), BOCHENEK (1978) and CROCKER (1979).

The present experiment was aimed at finding out whether the fat metabolism disturbance induced by us is reversible or not and how the increased lipid values become normalized if, to regenerate fat metabolism disturbance, a synthetic normal diet was fed containing either of the two fats in adequate (5%) quantity.

1. Materials and methods

In accordance with our previous experiment (J. N. ZSINKA et al., 1988b) groups of 40 sexually mature male Wistar rats of 220 ± 12 g body mass were fed our synthetic lipogenic diet (J. N. ZSINKA et al., 1988a) containing 20% sunflower-oil or a mixture of sunflower-oil and MCT C_8C_{10}-rich oil in the proportion of 33% : 67%, for 6 weeks. To generate fat metabolism distur-

Table 1

Composition of diet
Fat metabolism disturbance
(feeding for 6 weeks)

	Groups		
	I	II	III
	Normal rat diet	Synthetic lipogenic diet	
		sunflower-oil	oil mixture
Fat content (%)	5	20	20
Total saturated fatty acids (%) (S)	—	13.4	72.1
Polyunsaturated fatty acids (%) (P)	—	70.0	21.4
P/S	—	5.2	0.3
MCT (%)	—	—	67.7
Joule per 100 g diet	1600	1737	1726
Calorie per 100 g diet	385	417	415

Regeneration period
(postfeeding for 3 weeks)

	Normal rat diet	Synthetic lipogenic diet	
		sunflower-oil	oil mixture
Fat content (%)	5	5	5
P/S	—	5.2	0.3
MCT (%)	—	—	67.7
Joule per 100 g diet	1600	1598	1593
Calorie per 100 g diet	385	384	383

bance half of the rats were given, for further 3 weeks, a synthetic normal diet containing only 5% of the two kinds of fats. The control group was fed a normal rat chow.

Rats were fed isoenergetically (pair fed groups) and measured their actual consumption and the average energy uptake was calculated. Body mass of rats was weighed weekly. After the 6 weeks period of feeding with lipogenic diet, half of each group (20 rats) was given normal synthetic diet for 3 more weeks. In both cases blood was taken from 20 rats under ether narcosis. The organs of the animals (liver, heart, kidneys, spleen) were removed and weighed and the relative organ masses were calculated. The total lipid content of serum and liver extracted according to FOLCH and co-workers (1957) was determined by the method of ZÖLLNER and KRISCH (1962). The triglyceride content of the serum was determined by the Reanal extraction test, the total cholesterol and the HDL-cholesterol level, obtained by precipitation according to LOPEZ-VIRELLA and co-workers (1977), by Gödecke's enzyme test. The method of DOLE and MEINERTZ (1960) was used to determine the amount of the free fatty acids.

Ten rats of each group were used to determine the fatty acid pattern in the liver by gas chromatography. Group averages and the standard deviations were calculated. Analysis of variance (SACHS, 1984) was used to determine significant deviations.

2. Results

Diet consumption and energy uptake are shown in Table 2.

As visible, diet consumption and energy uptake were similar, and there was no difference in the regeneration period, either.

Table 2

Diet consumption and energy uptake of the rats

	Groups		
	I	II	III
Diet consumption g per 100 g body mass per day			
6 weeks	6.3	5.6	5.5
(regeneration) 3 weeks	6.1	6.3	6.4
J per 100 g body mass per day			
6 weeks	100	97	95
3 weeks	98.0	101.0	102.0
Cal. per 100 g body mass per day			
6 weeks	24	23	23
3 weeks	23.0	24.1	24.3

Table 3

Gain of body mass

	Groups		
	I	II	III
g per 6 weeks	134±16	178[a]±12	155 ±11
g per 3 weeks	17± 2	15 ±3	26[b]± 5

[a] = II — I, III P<0.05
[b] = I, II — III P<0.05

Relative mass of liver

	Groups		
	I	II	III
After 6 weeks	3.1±0.26	4.66[a]±0.33	4.27[ab]±0.35
After 3 weeks (regeneration)	2.9±0.09	3.4[ac]±0.36	3.2[c] ±0.47

[a] = I —II, III P<0.05
[b] = II —III P<0.05
[c] = 6 weeks — 3 weeks P<0.05

Gains in body mass and changes, which were observed in the relative liver masses only, are summarized in Table 3.

During the regeneration period the increase in body mass of the group consuming fat mixture (III) was significantly higher than that of the other two groups.

The relative liver masses increasing during the period of fat metabolism disturbance because of feeding on lipogenic diet, decreased significantly in both groups during the 3 weeks regeneration period when the animals were kept on synthetic normal diet. However, group II exceeded the control values.

The serum lipid values are summarized in Table 4.

During the 3 weeks regeneration period the increased serum lipid values of the first period were reduced. After consuming the fat mixture in the synthetic normal diet, the total lipid and total cholesterol content of the serum (III) were reduced to the level of the normal control values (I). As compared to the effect of sunflower-oil consumption (II) the total serum lipid and total cholesterol content were significantly lower after fat mixture consumption (III).

During the regeneration period the HDL_{chol} values did not show a definite tendency but the total cholesterol/HDL cholesterol index came near to the control values.

Table 4

Lipid levels of the serum

	Groups		
	I	II	III
Total lipid content (g dm^{-3})			
6 weeks	3.73 ±0.51	4.78[a] ±1.60	6.00[a] ±1.10
(regeneration) 3 weeks	3.47 ±0.40	4.21[a] ±0.90	3.50[b c] ±0.80
Triglyceride (mmol dm^{-3})			
6 weeks	0.76 ±0.20	0.53[a] ±0.13	0.49[a] ±0.15
3 weeks	0.55 ±0.15	0.38[a] ±0.17	0.51[b] ±0.15
Total cholesterol (mmol dm^{-3})			
6 weeks	1.94 ±0.35	2.89[a] ±0.69	5.29[a b] ±1.40
3 weeks	1.82 ±0.21	2.38[a] ±0.70	1.72[b c] ±0.71
HDL_{chol} (mmol dm^{-3})			
6 weeks	0.91 ±0.21	0.87 ±0.31	0.90 ±0.26
3 weeks	1.02 ±0.31	1.10 ±0.45	0.89 ±0.41
Free fatty acid (mmol dm^{-3})			
6 weeks	0.48 ±0.08	0.61 ±0.21	0.58 ±0.24
3 weeks	0.53 ±0.23	0.33[c] ±0.15	0.43 ±0.14
Total cholesterol/HDL_{chol}			
6 weeks	2.13 ±1.76	3.32[a] ±2.00	5.87[a b] ±2.12
3 weeks	1.78 ±1.62	2.16[c] ±1.15	1.93[c] ±1.19

[a] = I—II, III $P < 0.05$
[b] = II—III $P < 0.05$
[c] = 6 weeks — 3 weeks $P < 0.05$

Table 5 shows the lipid content of the liver.

Lipid values except the free fatty acid content increasing as the effect of lipogenic diet, were significantly reduced during the 3 weeks of synthetic normal diet consumption. The total cholesterol content of the liver decreased more substantially after the intake of 5% fat mixture in the normal diet than after sunflower-oil consumption. Substantial changes were not observed in other values upon consumption of the different fats.

Distribution of fatty acid in the liver is shown in Table 6.

During the 3 weeks regeneration period almost all the changed fatty acid values approached the control values, those of rats fed on mixture con-

Table 5

Lipid content of the liver

	Groups		
	I	II	III
Total lipid content (mg per g)			
6 weeks	79.4 ±11.0	369.0[a] ±102	308.0[a] ± 60
(regeneration)			
3 weeks	76.0 ±13.0	221.0[a c] ± 75.0	183.0[a c] ± 75.0
Triglycerides (mg per g)			
6 weeks	14.8 ± 4.0	87.0[a] ± 11.0	72.0[a b] ± 16.0
3 weeks	21.2 ± 2.4	42.0[a c] ± 12.0	46.0[a c] ± 15.0
Total cholesterol (mg per g)			
6 weeks	7.0 ± 1.0	116.0[a] ± 35.0	97.0[a b] ± 17.0
3 weeks	4.9 ± 0.8	57.4[a c] ± 18.0	36.0[b c] ± 21.0
Free fatty acids (mmol per g)			
6 weeks	14.8 ± 6.2	8.5[a] ± 3.1	10.2[a] ± 4.0
3 weeks	13.5 ± 9.0	9.6 ± 4.0	9.4 ± 4.1

[a] = I—II, III $P<0.05$
[b] = II—III $P<0.05$
[c] = 6 weeks—3 weeks $P<0.05$

taining diet more so than those fed on sunflower-oil-containing diet. This was most striking in the linoleic/arachidic acid ratio as well as in the fact that the P/S ratio of the liver was similar to the control ratio.

3. Conclusions

It is well known that the sunflower-oil (BOCHENEK, 1978; PFLUGRADT et al., 1981) and MCT-rich oil (LEVEILLE, 1967; STEWARD, 1978 and others) decrease certain serum lipid levels and as an effect of MCT C_8C_{10}-oil the lipid synthesis is low (DEMARNE, 1977). Therefore we aimed at examining the role of mixture of MCT C_8C_{10}-oil and sunflower-oil in the ratio of 67% : 33%, in comparison to the effect of a normal rat chow and a sunflower-oil-containing diet during the regeneration period after a diet-induced fat metabolism disturbance.

In the present experiment, serum and liver lipid values revealing fat metabolism disturbance induced by our lipogenic diet (J. N. ZSINKA, 1988a) were equal to the results of our former study and discussed there (J. N. ZSINKA,

Table 6

Percentage distribution of the fatty acids in the liver

Group diets	C_8	C_{10}	C_{14}	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:1}$	$C_{20:4}$	$C_{22:6}$
Fatty acids											
I Normal rat chow (6 weeks)	—	—	0.6 ±0.2	21.3 ± 2.8	4.4 ±2.2	19.4 ± 3.7	20.5 ± 2.3	13.6 ± 2.5	3.6 ±2.7	14.8 ± 2.1	1.8 ±1.9
II Lipogenic diet (6 weeks)	—	—	0.4 ±0.3	9.4 ± 3.0	2.5 ±0.4	3.4 ± 3.4	27.9 ± 2.9	47.0 ± 4.9	0.4 ±1.1	4.3 ± 1.0	4.3 ±2.5
Synth. normal diet (3 weeks)	—	—	0.6 ±0.3	15.9 ± 2.2	4.7 ±0.3	7.3 ± 3.9	28.2 ± 5.8	30.8 ± 8.1	2.7 ±2.2	7.7 ± 3.8	2.0 ±3.5
III Lipogenic diet (6 weeks)	0.5 ±0.3	0.4 ±0.2	1.6 ±1.1	17.4 ± 3.6	6.7 ±1.0	3.1 ± 2.2	34.7 ± 3.2	18.8 ± 6.4	4.5 ±2.7	7.5 ± 3.7	5.1 ±2.2
Synth. normal diet (3 weeks)	0.2 ±0.4	0.2 ±0.1	1.00 ±0.09	18.5 ± 4.2	6.7 ±2.7	14.1 ± 9.7	25.6 ± 6.2	19.6 ± 7.0	2.0 ±1.5	11.3 ± 7.0	1.3 ±2.0

Group diets	Total unsaturated (TU)	Mono-unsaturated (U)	Poly-unsaturated (P)	Saturated (S)	C_8 C_{10} MCT
I Normal rat chow	58.5	28.4	30.1	41.4	
II Lipogenic diet	86.5	30.9	55.6	13.2	
Synth. normal diet	76.1	35.6	40.5	23.8	
III Lipogenic diet	77.0	45.6	31.4	23.0	0.86
Synth. normal diet	66.6	34.3	32.2	33.0	0.39

Group diets	TU/S	P/S	P/U	TU/S	U/S	$C_{18:2}/P$	$C_{18:2}/C_{20:4}$
I Normal rat chow	1.4	0.7	1.0	1.4	0.7	0.4	0.9
II Lipogenic diet	6.5	4.2	1.7	6.5	2.3	0.8	10.8
Synth. normal diet	3.2	1.7	1.1	3.2	1.4	0.7	4.0
III Lipogenic diet	3.3	1.3	0.7	3.3	1.9	0.6	2.5
Synth. normal diet	1.9	0.9	0.9	1.9	1.0	0.6	1.7

1988b). The regeneration brought about by 3 weeks postfeeding on a synthetic normal diet containing 5% fat caused lipid indices to improve. Thus, fat metabolism disturbance and the developed "alimentary steatosis", the elevated serum and liver lipid contents proved to be reversible in a short time.

During the 3 weeks normal synthetic diet consumption the feeding of the fat mixture (III) significantly reduced the diet-induced higher total serum lipid values and above all, the total cholesterol contents. Sunflower-oil (II) did not cause further significant change in the serum values. Change in the liver lipids were more extensive; both kinds of fat caused a substantial reduction in all the lipid indices, except in free fatty acids. As an effect of the fat mixture a significantly lower level occurred in the total cholesterol content of the liver than upon sunflower-oil consumption. This is brought about partly by the lower unsaturated fatty acid content of the fat mixture (unsaturated fatty acids increase the triglyceride and cholesterol contents of the liver; TRISCARI, 1978; CROCKER, 1979; J. N. ZSINKA et al., (1987a, b; 1988a), and partly, by the MCT-oil component of the fat mixture, consisting of C_8 and C_{10} fatty acids, but no C_{12}. Namely according to the results of DEMARNE (1977), C_{12} fatty acids integrated in large amounts in the triglycerides or converted into C_{14} get metabolized, while fatty acids of lower carbon number are not transformed. An increased cholesterol turnover may be supposed, too.

As an effect of an adequate postfeeding, changes in the fatty acid content of the liver suggest regeneration of fat metabolism disturbances. Consumption of both kinds of fats caused the indices to approximate the normal control values. This is due mainly to the appropriate composition of the diet. However, the quality of the fat caused differences, too. Analysis of the fatty acid pattern of the liver has shown that the pattern, the proportion of essential fatty acids, came nearer to the normal values upon fat mixture consumption than upon one-sided sunflower-oil intake even in adequate quantity. The difference was most striking in the linoleic–arachidic acid ratio, showing that in fat metabolism disturbance the high amount of linoleic-acid present in the liver is not accompanied by conversion into arachidic acid. Similarly, NAIR (1981) shows that as an effect of a coconut oil based diet the change in linoleic acid content is higher, than that in arachidic acid level. Fatty acid results show a good correlation with the lipid values of the liver; in the regeneration period both indices approximated more the control values upon fat mixture consumption. The results of the present study indicate that the intake of an adequate fat mixture is of importance not only in prevention of fat metabolism disturbance but in the regeneration period as well, even at a reduced fat and normal diet consumption.

In relation to the quality of fats consumed it was established in earlier experiments (J. N. ZSINKA et al., 1987b) that beside their P/S ratio, other parameters, such as length of carbon chain or/and the quality of their fatty

acids of different length carbon chains play an important role. The present study had similar results: consumption of a fat mixture of P/S = 0.3 ratio, differing from the optimal ratio P/S = 1, containing fatty acids of C_8 and C_{10} chain length during the regeneration period nearly normalized all the lipid indices indicating disturbance of fat metabolism.

The results permit the conclusion that this model experiments may be suitable for the dietetic and therapeutic treatment of fat metabolism disturbance and for the determination of adequate fat consumption to prevent the development of the disease.

Literature

BOCHENEK, W. (1978): Effect of saturated and unsaturated fats given with and without dietary cholesterol. *Biochem. Biophys. Acta, 528*, 1–16.

CROCKER, P. J. (1979): Effect of the unsaturation of dietary fat and of arachidonate supplementation on cholesterol pool expansion in the Guinea pig. *J. Nutr., 109*, 927–932.

DEMARNE, Y. (1977): Effect of dietary lipids on qualitative aspects of lipogenesis in growing rat and pig. *Am. Biochem. Biophys., 17*, 875–882.

DOLE, V. P. & MEINERTZ, H. (1960): Microdetermination of long chain fatty acids in plasma and tissues. *J. Biol. Chem., 235*, 2595–2598.

FOLCH, J., LEES, M. & STANLEY, S. G. H. (1957): A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem., 226*, 497–509.

J. N. ZSINKA, Á., FÖLDES, V. & PERÉDI, J. (1987b): Certain serum and liver lipid parameters as affected by different fat mixtures. *Acta Alimentaria, 16*, 225–230.

J. N. ZSINKA, Á., FÖLDES, V. & PERÉDI, J. (1987a): Certain serum and liver lipid parameters as affected by edible fats of different quantity and quality. *Acta Alimentaria, 16*, 217–223.

J. N. ZSINKA, Á., FÖLDES, V. & PERÉDI, J. (1988a): Indices of lipid metabolism disturbance induced by diet. *Acta Alimentaria, 17*, 43–51.

J. N. ZSINKA, Á., FÖLDES, V., GERE, A., SZÉPVÖLGYI, J., PERÉDI, J. & THURÓCZY-THIERMAYER, T. (1988b): Influence of dietary oils in experimental lipid metabolism disturbance. *Acta Alimentaria*, 17, 211–218.

LEVEILLE, G. A. (1967): Influence of medium chain triglycerides on lipid metabolism in the rat. *Lipids, 2*, 287–295.

LOPEZ-VIRELLA, M. F., STONE, P. G., ELLIS, S. & COLLWELL, J. (1977): Cholesterol determination in high density lipoproteins separated by three different methods. *Clin. Chem., 23*, 882–884.

NAIR, P. G. V. (1981): Effect of long term feeding of a coconut oil based diet on serum cholesterol and fatty acid composition of different organs of albino rats. *J. Fd Sci. Technol., 18.*, 187–191.

PFLUGRADT, K., VOSS, CH., HARTMANN, N. & RATZMANN, L.-K. (1981): Zum Einfluss einer Fettdiät auf dem Gehalt an Gesamtcholesterol, freiem Cholesterol und Estercholesterol in Serum und Leber von Ratten. *Nahrung, 25*, 937–941.

SACHS, L. (1984): *Applied statistics.* A handbook of techniques. Springer Verlag, Berlin.

STEWARD, J. W. (1978): Effect of various triglycerides on blood and tissue cholesterol of calves. *J. Nutr. 108*, 561–569.

TRISCARI, J. (1978): Comparative effect of saturated and unsaturated lipids on hepatic lipogenesis and cholesterogenesis. *J. Nutr., 108*, 815–825.

ZÖLLNER, N., & KRISCH, K. (1962): Über die quantitative Bestimmung von Lipoiden (Micromethode) mittels der vielen natürlichen Lipoiden. *Z. ges. exp. Med., 135*, 545–562.

Acta Alimentaria, Vol. 18 (1), pp. 13—18 (1989)

COMPOSITION AND AMINO ACID PROFILES OF TOMATO SEEDS FROM CANNING WASTES

P. R. CANTARELLI, E. R. PALMA and J. G. B. CARUSO

Department of Rural Technology, College of Agriculture, University of São Paulo. P. O. Box 9, 13400 Piracicaba, Brazil

(Received: 15 July 1987; revision received: 10 June 1988; accepted: 13 June 1988)

Tomato seeds from processing wastes and previously submitted to Hot and Cold Break treatments were demonstrated to be a potential source of nonconventional protein due to high lysine contents even though showing primary limitation for valine. The effect of such thermal treatments on the protein quality, chemical composition and amino acid profiles was observed.

Valine limitation; protein; fat; carbohydrates; fiber and ash contents were: 67 and 74; 33.71 and 36.77; 14.62 and 19.00; 4.76 and 5.35; 41.78 and 33.04; 2.01 and 2.14% for seeds from Hot and Cold Break treatments, respectively. However, due to high fiber content tomato seed meals showed to be more suitable for isolation of protein concentrates.

Keywords: tomato seed protein, tomato seed wastes

The disposal of many cannery wastes usually involves technical, economic and environmental problems. Tomato processing wastes consisting primarily of seeds and skins are often buried, flushed into rivers and streams (KELLEY, 1958) or used as animal feed (EDWARDS et al., 1952; AMMERMAN et al., 1963; BEN-GERA & KRAMER, 1969; KRAMER & KWEE, 1977b). The composition of and isolation of protein concentrate from tomato seeds have been studied by several researchers (BEN-GERA & KRAMER, 1969; TSATSARONIS & BOSKOU, 1975; KRAMER & KWEE, 1977a; CANELLA et al., 1979; CANELLA & CASTRIOTTA, 1980; BRODOWSKI & GEISMAN, 1980; LATLIEF & KNORR, 1983a, b).

The expanding tomato processing industry yields a considerable amount of seeds that could be converted into high protein supplements for an undernourished population. Such conversion would decrease the shortage of high quality protein, and at the same time, help to minimize pollution risks, mainly in developing countries. The purpose of this work was to evaluate the composition of tomato seed wastes submitted to industrial thermal treatments.

Akadémiai Kiadó, Budapest

1. Materials and methods

1.1. Preparation of dry defatted seed meals

Samples of tomato pomace were obtained after commercial extraction of ripe tomatoes (cultivar Petomech AG 16) grown in São Paulo, Brazil, which were submitted either to Hot Break (95 °C 10 min^{-1}) or Cold break (60 °C 10 min^{-1}) treatments. The samples, after a spontaneous fermentation at room temperature were washed with tap water to separate seeds from skins and gelatinous material. The seeds were dried at 45 °C for 48 h in a forced air oven, comminuted with a grinder model 4-E (Quaker City Mill, Pa.) and defatted with n-hexane for 6 h in a Soxhlet apparatus, following by a regrinding in a hammer mill fitted with a 40 mesh screen.

1.2. Analytical methods

1.2.1. *Crude protein.* Total nitrogen content was determined by micro-Kjeldahl analysis following the method as described by BAILEY (1967) and crude protein expressed as N(%) $\times 5.85$.

1.2.2. *Amino acids.* Appropriate amounts of defatted seed meals were hydrolyzed in sealed tubes at 110 °C for 24 h with 6 mol l^{-1} HCl. The excess of hydrochloric acid was evaporated under vacuum, with occasional addition of distilled water 3–5 times. The residue was dissolved in 10% isopropanol (v/v) then filtered and the final volume made up to 5 cm^3 in a measuring flask with the same solvent. Methionine and cystine were analyzed after oxidation with performic acid by the procedure of MOORE (1963). The percentage of each amino acid was determined in a Beckman model 120 C automatic amino acid analyzer, following the technique described by SPACKMAN and co-workers (1958). Tryptophan was determined according to MERTZ and co-workers (1975).

1.2.3. *Total carbohydrates.* Carbohydrates were extracted with 85% methanol (v/v) (SOUTHGATE, 1969) and determined with anthrone reagent (MORRIS, 1948).

1.2.4. *Other analyses.* Moisture, crude fat and ash were determined according to standard methods (AOAC, 1980). Crude fiber was estimated by difference. The nutritional quality of tomato seed meal proteins was evaluated by their chemical scores.

1.2.5. *Statistical analysis.* Mean values of results, standard deviations and comparison of mean values (F test) between components of three samples for each thermal treatment, were calculated with four replicates, except for amino acid analyses which were performed in duplicate on a composite of the three original samples per treatment.

2. Results and discussion

The proximate composition of tomato seed meals is shown in Table 1. Average crude protein ranged from 33.71% to 36.77% for seeds submitted to Hot and Cold Break treatments, respectively. This fact indicates that the high temperature of the Hot Break treatment resulted in more noticeable losses of nitrogen compounds of tomato seeds. The data obtained for both treatments were lower than those reported by ANTALDI and PIVA (1967); VIGO and co-workers (1977); CANELLA and CASTRIOTTA (1980) or MORAD and co-workers (1980), but higher than those obtained by TSATSARONIS and BOSKOU (1975); CANELLA and co-workers (1979); BRODOWSKI and GEISMAN (1980) or RAKHMETOVA (1980).

Ash and fat contents were found to be lower than the results observed by other authors (TSATSARONIS & BOSKOU, 1975; CANELLA et al., 1979) but quite similar to the data presented by CANELLA and CASTRIOTTA (1980). While ash was not affected by the temperature the lower fat content of seeds submitted to Hot Break treatment showed such influence. The same effect of high temperature was demonstrated by the lower carbohydrates in the Hot Break seed meals.

Although crude fiber was estimated by difference, the high values observed could impair the use of tomato seed meals as a food supplement. As a consequence these by-products may be more suitable for obtaining pure protein isolates.

The amino acid profiles are given in Table 2. The amino acid composition, for both treatments, is similar to the data reported by TSATSARONIS

Table 1

Effect of industrial thermal treatments on the composition of tomato seed meals (g per 100 g of dry matter)

Component	Hot Break		Cold Break		Level of significance
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	
Moisture	3.11	0.07	3.70	0.02	**
Crude protein	33.71	0.18	36.77	0.42	**
Total carbohydrates	4.76	0.19	5.35	0.05	**
Crude fat	14.62	0.19	19.00	0.10	**
Crude fiber[a]	41.78	0.11	33.04	0.43	**
Ash	2.01	0.00	2.14	0.02	ns

[a]: estimated by difference
$\bar{x}$: mean value
$\pm s$: standard deviation
ns: not significant
**: significant at P = 1% probability level
Number of measurements, (n) = 4

Table 2

Amino acid composition of tomato seed as affected by industrial thermal treatments

Amino acid	Hot Break meal	Cold Break meal	Change due to thermal treatment (%)
	(g amino acid per 100 g protein)[a]		
Lysine	8.18	8.41	— 2.73
Histidine	3.61	4.04	—10.64
Arginine	9.60	9.83	— 2.34
Aspartic acid	10.93	11.40	— 4.12
Threonine	3.25	3.99	—18.55
Serine	4.39	4.52	— 2.88
Glutamic acid	13.25	13.65	— 2.93
Proline	5.63	6.53	—14.18
Glycine	5.25	5.38	— 2.42
Alanine	3.52	3.62	— 2.76
Cystine	2.70	2.42	+11.57
Valine	3.69	4.43	—16.70
Methionine	2.58	2.33	+10.73
Isoleucine	3.84	4.51	—14.86
Leucine	5.66	6.01	— 5.82
Tyrosine	4.74	5.29	—10.40
Phenylalanine	4.74	5.77	—17.85
Tryptophan	1.16	1.26	— 7.94

[a]The duplicate samples presented values not exceeding a $\pm 5\%$ deviation according to the techniques used.

and BOSKOU (1975); CANELLA and co-workers (1979); CANELLA and CASTRIOTTA (1980); BRODOWSKI and GEISMAN (1980); LATLIEF and KNORR (1983a), except for the higher values found for lysine, histine, proline and cystine and for the lower value for glutamic acid. Lysine contents as reported

Table 3

Chemical Scores (CS) and A/E ratios of tomato seed meals in comparison with hen's egg protein

Amino acid	Egg protein A/E	Hot Break meal		Cold Breal meal	
		A/E	CS	A/E	CS
Isoleucine	110	95	86	101	92
Leucine	175	140	80	135	77
Lysine	143	202	141	189	132
Methionine + Cystine	116	130	112	107	92
Phenylalanine + Tyrosine	190	234	123	249	131
Threonine	96	80	83	90	94
Valine	135	91	67	100	74
Tryptophan	35	29	83	28	80

herein seem to be uncommon compared to the highest value (6.6 g per 100 g protein) reported by BRODOWSKI and GEISMAN (1980). This finding could be attributed to the fact of beside being under genetic control, protein can be greatly influenced by factors of cultivation and environment, such as availability of nitrogen to the plant (JOHNSON et al., 1972).

It is worthwhile to observe the trend that amino acid composition is affected by thermal treatment. Noticeable losses with Hot Break process occurred in relation to essential amino acids, threonine, valine, isoleucine and leucine. However, the unexpected retention of sulfur containing amino acids must be the objective of further studies.

Table 3 shows the A/E ratios and percentages of limitation compared to hen's egg protein (LAJOLO et al., 1982). While valine is limiting (67% and 74% for Hot Break and Cold Break meals, respectively) and somewhat deficient in other amino acids, tomato seed proteins could be used to improve the nutritive value of low lysine foods while they lack antinutritional factors (GEISMAN, 1981).

Despite several technological problems, such as seed drying or protein concentrate isolation, tomato seed wastes appear to be a promising and potentially valuable protein source for improving human diets, instead of simply being discarded.

3. Conclusions

The aforementioned results allow the following conclusions:

The temperature used in Hot Break treatment reduced the protein, fat and carbohydrate contents of tomato seed meals.

As a consequence of their high fiber content tomato seed meals are more suitable for obtaining protein isolates.

A trend that amino acid contents could be affected by heat was observed. In this respect further studies are required with sulfur containing amino acids.

Although some amino acids (mainly valine) are limiting, tomato seed protein would still be a promising source for supplementation of low lysine foods.

Literature

AMMERMAN, C. B., ARRINGTON, L. R., LOGGINS, P. E., MC CALL, J. T. & DAVIS, G. K. (1963): Nutritive value of dried tomato pulp for ruminants. *J. agric. Fd Chem., 11*, 347–349.

ANTALDI, G. G. V. & PIVA, G. (1967): Tenore in amoniacid dei sottoprodotti del pomodoro. *Ann. Fac. Agr. Univ. Catt. Sacro Cuore, 7*, 639–642.

AOAC (1980): Official methods of analysis. 14th ed., Association of Official Analytical Chemists. Washington D. C., p. 1018.

BAILEY, J. L. (1967): Determination of nitrogen. — in: BAILEY, J. L. (Ed.) *Techniques in protein chemistry.* Elsevier, Amsterdam, pp. 346–347.

BEN-GERA, I. & KRAMER, A. (1969): The utilization of food industries wastes. *Adv. Fd Res., 17*, 77–151.

BRODOWSKI, D. & GEISMAN, J. R. (1980): Protein content and amino acid composition of protein of seed from tomatoes at various stages of ripeness. *J. Fd Sci., 45*, 228–229, 235.

CANELLA, M., CARDINALLI, F., CASTRIOTTA, C. & NAPPUCCI, R. (1979): Caratteri chimici dei semi di pomodore di differenti varietà. *Riv. Ital. Sostanze Grasse, 56*, 8–11.

CANELLA, M. & CASTRIOTTA, G. (1980): Protein composition and solubility of tomato seed meal. *Lebensm. Wiss. u. -Technol., 13*, 18–21.

EDWARDS, P. W., ESKEW, R. K., HOERSCH, A. Jr., ACETO, M. C. & REDFIELD, C. S. (1952): Recovery of tomato processing wastes. *Fd Technol., 6*, 383–386.

GEISMAN, J. R. (1981): Protein from tomato seeds. *Ohio Report on Research and Development, 66*. Dep. Hort. Ohio State University, Columbus, OH, pp. 92–94.

JOHNSON, V. A., MATTERN, P. J. & SCHMIDT, J. W. (1972): Genetic studies of wheat protein. — in: Symposium: *Seed proteins*. AVI Publishing Co., Inc., Westport, Con., p. 126.

KELLEY, E. G. (1958): Plant residues and pomaces. — in: ALTSCHUL, A. M. (Ed): *Processed plant protein*. Academic Press Inc., New York, pp. 859–875.

KRAMER, A. & KWEE, W. H. (1977a): Functional and nutritional properties of tomato seed protein concentrate. *J. Fd Sci., 42*, 207–211.

KRAMER, A. & KWEE, W. H. (1977b): Utilization of tomato processing wastes. *J. Fd Sci., 42*, 212–215.

LAJOLO, F. M., SANTOS, A. C. & WILSON, E. D. (1982): Proteinas e aminoacidos. — in: *Nutricão Basica*, Sarvier, S. Paulo, p. 37.

LATLIEF, S. J. & KNORR, D. (1983a): Tomato seed protein concentrates: Effects of methods of recovery upon yield and compositional characteristics. *J. Fd Sci., 48*, 1583–1586.

LATLIEF, S. J. & KNORR, D. (1983b): Effect of chitin as coagulating aid on protein yield, composition and functionality of tomato seed protein concentrates. *J. Fd Sci., 48*, 1587–1590.

MERTZ, E. T., JAMBUNATHAN, R. & MISRA, P. S. (1975): *Simple chemical and biological methods used at Purdue University to evaluate cereals for protein quality*. International Programs in Agricultural Station. Bulletin No 70. Agronomy Dep. Purdue University, Lafayette, IN, pp. 1–25.

MOORE, S. (1963): On the determination of cystine as cysteic acid. *J. biol. Chem. 238*, 235–237.

MORAD, N. M., EL-TAMINI, A. H. RADY, A. H. & IBRAHIM, S. S. (1980): Tomato seed oil. — Part II. Evaluation of the Egyptian tomato seed oil and residual meal. *Fette, Seife Amstrich-Mittel, 3*, 122–124.

MORRIS, D. L. (1948): Quantitative determination of carbohydrates with Dreywood's Anthrone Reagent. *Science, 107*, 254–262.

RAKHMETOVA, S. R. (1980): Chemical composition of tomato seeds. *Voprosy Pitaniya 1*, 74. — ref.: *Fd Sci. Technol. Abstr., 13*, 74.

SOUTHGATE, D. A. T. (1969): Determination of carbohydrates in foods. — Part I. Available carbohydrate. *J. Fd Sci., Agric. 20*, 326–330.

SPACKMAN, D. H., STEIN, W. H. & MOORE, S. (1958): Automatic recording apparatus for use in chromatography of amino acids. *Analyt. Chem., 30*, 1190–1206.

TSATSARONIS, G. C. & BOSKOU, D. G. (1975): Amino acid and mineral salt content of tomato seed and skin wastes. *J. Sci. Fd Agric.*, 421–423.

VIGO, M. S., DASSO, I. & CATTANEO, P. (1977): Studies on the seeds remaining after the processing of tomatoes. *An. Acad. Cienc. exact fis. nat. B. Aires, 29*, 193–203.

Acta Alimentaria, Vol. 18 (1), pp. 19—30 (1989)

INVESTIGATION OF THE ADDITIVITY OF FLOUR CHARACTERISTICS IN TWO-COMPONENT WHEAT FLOUR MIXTURES

K. HORVÁTH-ALMÁSSY

College Faculty of Food Industry, University of Horticulture and Food Industry, H-6701 Szeged, Marx tér 7. Hungary

(Received: 5 August 1987; revision received: 11 January 1988; accepted: 13 January 1988)

Baking quality, pastry and protein properties of two-component wheat flour mixtures were tested in model experiments. The four basic flour samples comprised a wide range, almost three orders of baking quality (Jubilejnaja 50, MV 8, Baranjka and GK Csilla of the year 1986).

It was established that some of the characteristics additioned in the course of mixing means a high correlation between the properties as measured or calculated on basis of composition. Properties studied were: water absorption capacity, valorigraph value, SDS sedimentation value.

However, other properties (loaf volume, largest cross-section), important from the point of view of the final product, did not prove additive at all. Results corresponded highly with the experiences of BOLLING (1980).

The experiments permit the conclusion that the rheological properties of the pastry or the gluten of individual flours highly affect the baking quality of the mixture obtained from them. The mixture of a flour producing short, firm texture and another containing softened gluten may give a loaf of optimal volume although the quality of the two flours is far from optimal. The results point to the importance of the rheological properties of wheat true to variety for the baking industry. The deliberate utilization of the combinative capacity of different flours may improve the economicalness of the milling and baking industries while simultaneously satisfying the requirements of the consumer.

Keywords: wheat flour mixtures, baking quality, additivity of flour characteristics

The processing of wheat is an economic activity aimed at producing from the available raw material the best possible product at the lowest possible cost.

A heterogeneity of quality is characteristic of the wheat varieties in general cultivation. This is brought about mainly by the fact that the varieties giving a high yield and resistant to plant diseases have a lower baking quality while the varieties of high baking quality have a lower yield. Due to the frequently observed approach oriented at high yield the edible wheat varieties produced by the agriculture differ in their composition and structural characteristics.

The baking industry, in order to be able to produce bread of uniform quality, needs flour of uniform quality. Only meeting this requirement makes possible the optimization of technical processes.

It is an observation of old standing that by mixing the proper varieties a flour blend meeting the above requirement can be produced. The mixing

 Akadémiai Kiadó, Budapest

process, preconditioned by great experience and expertise, begins during milling while the final composition is formed in the bakery.

The baking quality of the flour used for bread baking is determined by the wheat varieties used for mixing. By analysing the properties of pastry and protein though the interplay of the factors in flour mixtures it becomes possible to gain a deeper knowledge of the effect of blending.

In the experiments described below the mutual effect of the mixed flours upon their baking quality, was studied in two-component model system.

1. Materials and methods

1.1. Materials

Four wheat samples of highly differing baking quality and their mixtures were used in the experiments. The basic flours were obtained from the Cereal Research Institute, Szeged and originated from the 1986 harvest. The wheat varieties used were: Jubilejnaja 50, Martonvásári 8 (MV 8), Baranjka and GK Csilla.

The two component mixtures were composed of Type BL 55 flours of the four basic wheat varieties applying the following proportions: 0 : 100, 25 : 75; 50 : 50; 75 : 25; 100 : 0 w/w.

Table 1

Properties studied of the four basic flour samples

Property	Wheat variety			
	Jubilejnaja 50	MV 8	Baranjka	GK Csilla
Loaf volume (cm^3)	1200	910	930	850
Largest cross section (cm^2)	114.3	98.0	98.7	90.1
Valorigraph value	82.7	60.4	65.2	46.0
Type of quality	A_2	B_1	B_1	B_2
Water binding capacity (%)	65.0	62.0	58.6	63.4
Quantity of wet gluten (%)	33.5	33.0	30.5	37.0
Gluten spreading (mm)	4.0	5.0	3.5	11
Raw protein content (%)	14.38	11.67	11.88	14.38
SDS sediment volume (cm^3)	6.5	5.1	6.0	4.1
Moisture content (%)	10.8	10.81	10.85	10.69
Ash (%)	0.562	0.451	0.471	0.450

The wheat samples were ground on the laboratory mill (manufactured by ÉLGÉP, Budapest) of the Research Institute of the Milling Industry. The total number of samples was 22 of which 4 were those of the basic wheat varieties and 18 of their two-component mixtures.

1.2. Methods

To characterize the baking quality of the 22 flour samples the following parameters were determined, according to KARÁCSONYI (1970):

1.2.1. Test loaf: volume, largest cross section, shape quotient.

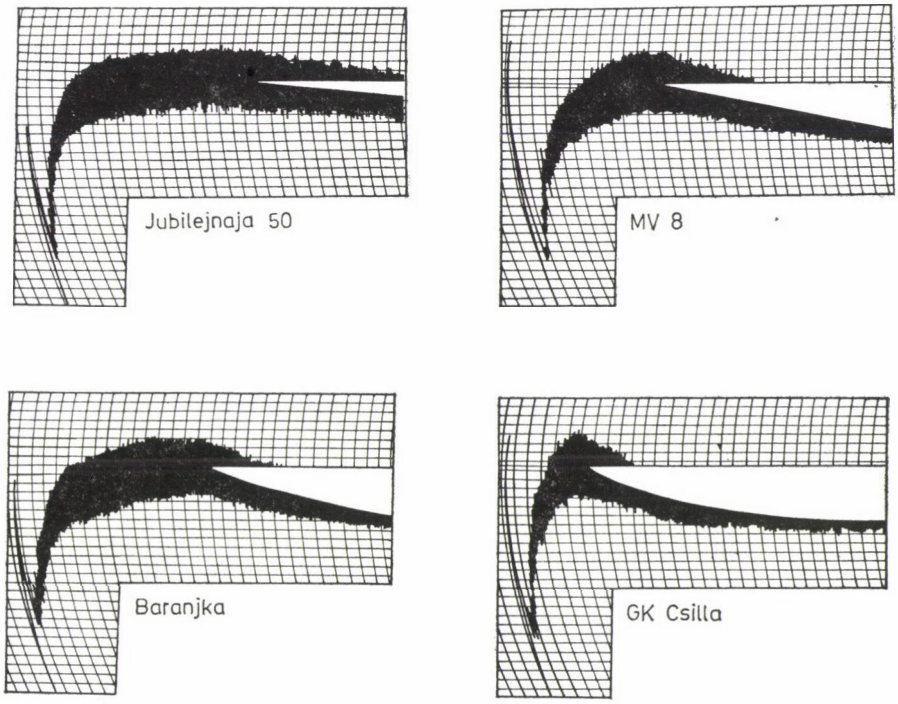


Fig. 1. Valorigrams of the basic wheat varieties

	Jubilejnaja 50	MV 8	Baranjka	GK Csilla
Water absorption capacity (%)	65.0	62.0	58.6	63.4
Formation (min)	5.5	4.0	6.0	2.7
Stability (min)	4.0	1.4	1.8	0.5
Softening	40	110	100	120
Softened area (cm^2)	1.9	9.5	7.4	17.0
Value	82.7	60.4	65.2	46.0
Quality group	A_2	B_1	B_1	B_2

1.2.2. Valorigraph test: baking value, water absorption capacity.

1.2.3. Gluten characters: wet gluten, expansion of gluten.

1.2.4. Protein characters: total protein content, SDS sedimentation value.

The test loafs were baked in the Control Laboratory of the Bakery Enterprise of County Csongrád. The SDS test, also characteristic of baking quality was carried out at the Kiszombor Plantation of the Cereal Research Institute, Szeged with the Lelley-type Sedimentor by the Axford method as modified by PALLAGI and MATUZ (1984). Determinations were carried out according to the related valid standards.

2. Results

Characteristic data o the four basic flours are given in Table 1. The four samples comprised a wide range of valorigraph values from the high quality A_2 to the poor quality B_2. It is interesting to note that the best sample of the four, Jubilejnaja 50 and the poorest, GK Csilla both contained 14.38% raw protein, while the other two varieties of medium quality 3% less. Thus, the total amount of protein in itself does not determine the baking quality.

Figure 1 shows the valorigrams of the four basic flour samples and the most important properties of the flours, readable.

The valorigrams give a good illustration of the character, from the point of view of baking quality, of each of the four flours.

It is worth mentioning that the wet gluten content of the samples, which has a determinative importance in the new system of wheat inspection

Table 2

Range of parameter values of the samples studied

Parameter	Range of values			
	average	minimum	maximum	scatter
Loaf volume (cm^3)	1006	850	1200	111
Largest cross section (cm^2)	104.8	90.1	117.0	5.0
Shape quotient	1.99	1.61	2.36	0.17
Valorigraph value	63.8	43.2	82.7	11.8
Water absorption capacity (%)	62.1	58.2	65.0	2.0
Wet gluten quantity (%)	34.2	30.5	38.5	2.1
Gluten spreading (mm)	5.1	2.5	11.0	2.3
Raw protein content (%)	12.32	11.67	14.38	1.06
SDS sediment volume (cm^3)	5.50	4.13	6.53	0.80

Number of measurements (n) = 22

in Hungary, does not show direct correlation to baking quality. As it can be seen, the poorest of the flour samples, GK Csilla, has the highest wet gluten content.

The parameters of all the samples studied are given in Table 2.

3. Discussion

3.1. Analysis of correlations between individual flour properties

Considering each sample as an independent unit, in the model system of mixed flour of 22 different flours, a simple linear regression calculation was carried out.

The correlations are expressed by the correlation matrix (Table 3).

The close correlation between the valorigraph value and the SDS sediment volume is of particular interest. The regression line is shown in Figure 2.

The regression curve is described by equation $y = 1.49 + 0.0629\ x$ ($r = 0.947$; n = 22).

This close correlation is again a proof of the fact that the resistance of the dough to mechanical stress, a property very important in bread baking, can be well characterized by the SDS sedimentation test.

It is remarkable, however, that the correlation between loaf volume, characterized by the largest cross section, and the valorigraph value is very poor, nonsignificant ($r = 0.509$, $r = 0.413$) in this series of experiments.

3.2. Analysis of flour properties' additivity by simple linear regression calculation

Milling and baking experiences show that by mixing different wheat varieties or flours of different quality the mixture obtained is of better baking quality than expected or calculated on the basis of composition (BOLLING 1977, 1983).

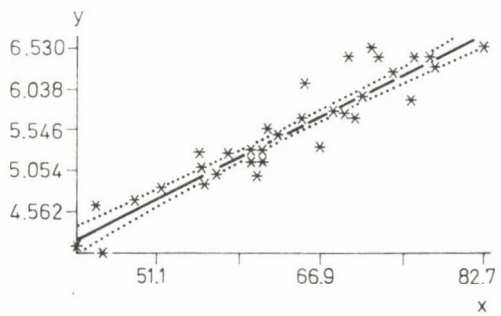


Fig. 2. Correlation between valorigraph value *(x)* and SDS sedimentation value *(y)*

Table 3

Correlation between the parameters studied

Parameter	Loaf volume	Largest cross section	Shape quotient	Valorigraph value	Water absorption capacity	Wet gluten	Gluten expansion	Raw protein	SDS sediment volume
Loaf volume	1	0.812***	0.348	0.509	0.574**	—0.085	—0.194	0.682***	0.390
Largest cross section		1	0.157	0.417	0.413	—0.032	—0.259	0.488	0.368
Shape quotient			1	—0.228	0.536**	0.367	0.542**	0.648**	0.157
Valorigraph value				1	0.182	—0.681***	—0.790***	—0.056	0.948***
Water absorption capacity					1	0.310	0.248	0.752***	0.084
Wet gluten						1	0.668***	0.332	—0.756***
Gluten spreading							1	—0.434	—0.834* **
Raw protein								1	—0.133
SDS sediment volume									1

** highly significant at P = 1% probability level
*** very highly significant at P = 0.1% probability level

Table 4

Investigation of the additivity of individual flour properties measured by simple linear correlation calculation

Parameter	r	Coefficients		$\frac{a}{y_{max}} 100$	(1—b) 100
		a	b		
Loaf volume	0.594**	385.6	0.6447	32.1	35.53
Largest cross section	0.352	79.09	0.2689	67.6	73.11
Shape quotient	0.373	0.254	0.8427	10.8	15.73
Valorigraph value	0.951***	—11.10	1.1700	13.4	17.00
Water absorption capacity	0.952***	—2.910	1.0400	4.48	4.00
Wet gluten	0.652**	7.100	0.8126	18.4	18.74
Gluten spreading	0.929***	—0.2942	0.8840	2.68	11.60
Raw protein	1.000	0.0000	1.0000	0.00	0.00
SDS sediment volume	0.886***	0.1475	0.3891	2.3	1.09

** highly significant at P = 1% probability level
*** very highly significant at P = 0.1% probability level
Number of measurements (n) = 18

To be able to decide which of the parameters of the 18 two-component mixtures may be considered additive a simple linear correlation was calculated on the basis of the proportion of components between the calculated values and those actually measured. Whether a property can be considered additive or not can be judged by the closeness of the correlation coefficient, the a constant and b slope of the regression line (Table 4).

The correlation coefficients show close correlation between the calculated and measured valorigraph value ($r = 0.951$), between the calculated and measured water binding capacity ($r = 0.952$) and between the calculated and measured values of gluten spreading ($r = 0.929$). A lower correlation, however, still significant at the P = 1% probability level, was found between the calculated and measured values of the SDS sediment volume ($r = 0.886$).

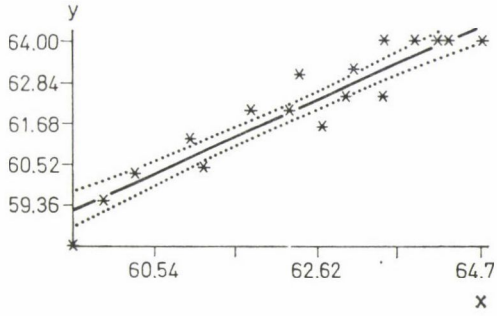


Fig. 3. Correlation between water absorption capacity as calculated *(x)* based on composition and as measured *(y)* by valorigraph

The property investigated is considered additive if in addition to close correlation the regression curve deviates only slightly from line $y = x$. The nearer constant a is to 0 and b quotient to 1 the surer that additivity exists in addition to close correlation.

In the last two columns of Table 4 the percentage deviations of a and b from 0 and 1, respectively, are shown. Additivity was found to be highest with water absorption capacity, SDS sediment volume expansion of gluten

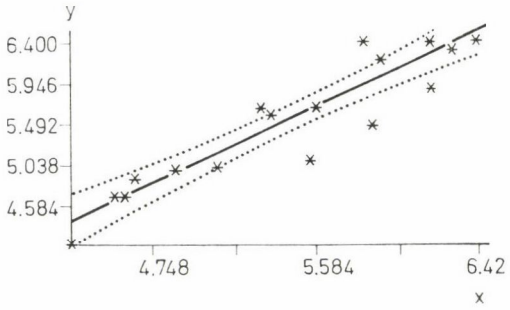


Fig. 4. Correlation between SDS sedimentation value as calculated *(x)* and as measured *(y)*

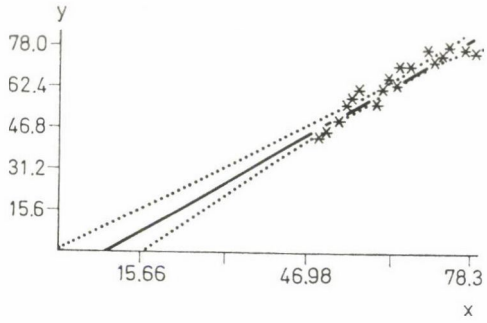


Fig. 5. Regression of the valorigraph value as calculated *(x)* and as measured *(y)*

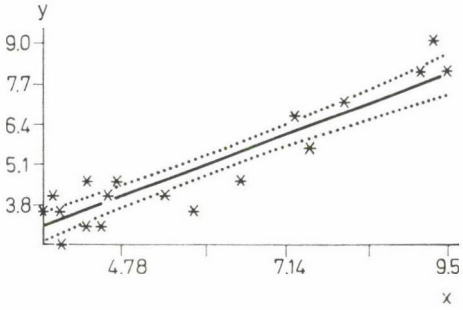


Fig. 6. Correlation between the calculated value *(x)* and the measured value *(y)* of gluten spreading

and valorigraph value. The regression curves are shown in Figures 3, 4 and 5. With gluten spreading most of the points were found to be outside the confidence region (Fig. 6).

It is worth noting that the correlation coefficient was low in the case of loaf volume or of the largest cross section, a quality characteristic of equal value, while the deviation of the regression curve from line $y = x$ was highly significant.

These results permitted the conclusion that in contrast to the water absorption capacity, the valorigraph value as well as the SDS sediment volume, the loaf volume and the largest cross section, in high correlation with the former, does not change additively in the mixture.

3.3. The non-additive flour parameters as functions of composition

Of the flour parameters proved non-additive, referred to the results given in para. 3.2., the most deviating cross section values are shown in pairs of mixtures as a function of composition (Fig. 7).

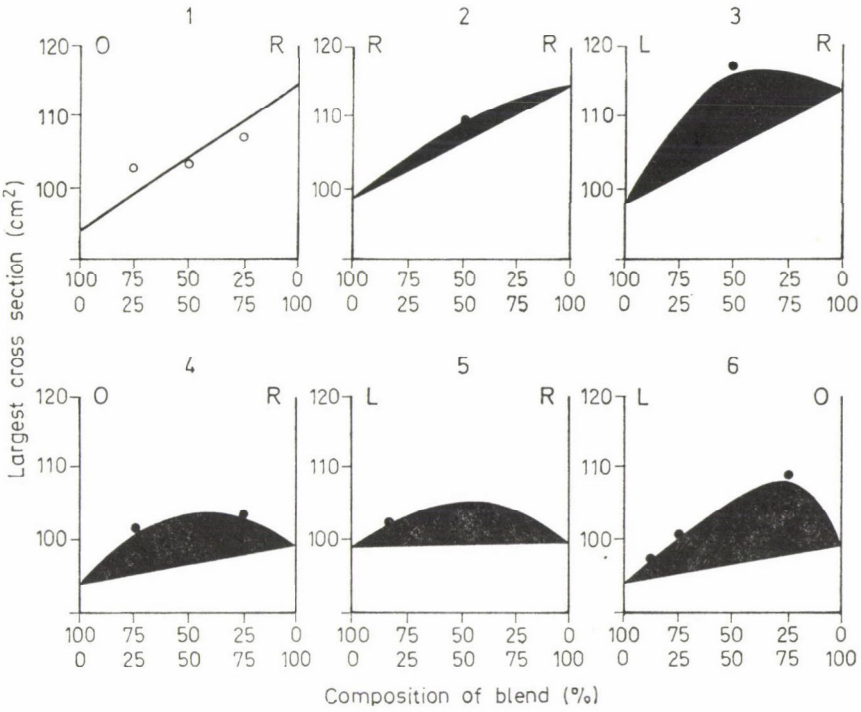


Fig. 7. Imprint of the test loaf as a function of composition
Composition: 1. MV 8 — Jubilejnaja 50; 2. Baranjka–Jubilejnaja 50; 3. GK Csilla–Jubilejnaja 50; 4. MV 8–Baranjka; 5. GK Csilla–Baranjka; 6. GK Csilla–MV 8
Spreading of gluten: R: short, firm; O: optimal, normal; L: soft, long

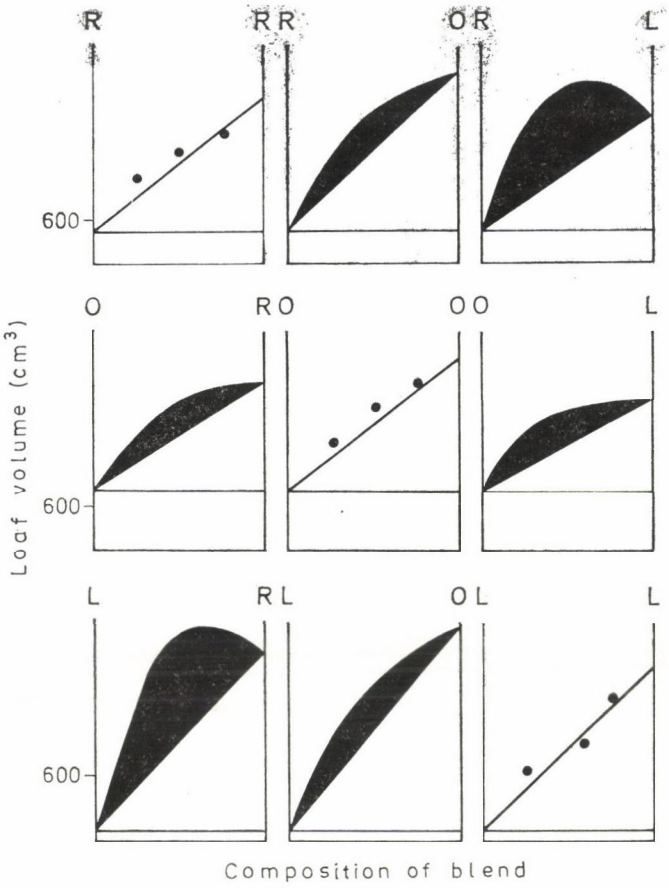


Fig. 8. Prototypes of the combination effects on dough character of short, normal and soft wheat varieties (BOLLING, 1980)
Rheological characters of dough: R: short, firm; O: optimal, normal; L: soft, long

The majority of the curves are of maximum character, meaning that to some of the compositions belonged maximum loaf volumes. Exceptions were mixture types 1 and 2 where simple additivity manifested itself.

Extreme deviation from the calculated value appeared in the case of combinations 3 and 6.

To be able to evaluate this phenomenon these figures were compared to those published by BOLLING (1980) under the title: Prototypes of mutual effects upon combination of wheat varieties giving short, normal or soft dough (Fig. 8).

Evident similarities were observed. On studying the effect of combination of different wheat varieties BOLLING (1980) found that by mixing flour of synergistic dough rheological characteristics extremely advantageous

changes in dough structure can be achieved. For instance, when a variety with particularly soft gluten is combined with a short firm dough forming variety, a highly improved product can be obtained.

The improving effect can be defined as an increase of loaf volume brought about by the added component through optimization of the dough rheological properties.

In bakery practice three types of dough are distinguished: normal or optimal, firm and soft. Among other methods the viscoelastic properties of the dough may be characterized by the spreading of gluten.

On the basis of the spreading test the four flours used in these experiments can be classified in the following types:

Jubilejnaja 50	short, firm,
MV 8	normal (at the lower margin),
Baranjka	short, firm,
GK Csilla	soft.

Every one of the diagrams presented in Fig. 7 was comparable to one of the basic types according to BOLLING (1980), except No. 1. However, the gluten characters of these flour mixtures of two components were fairly similar (MV 8–Jubilejnaja 50).

Naturally the shape of the diagrams was affected by the loaf volume of the original flours.

It is clearly visible that when a wheat variety giving a firm texture is mixed with another variety of the same character, the increase of loaf volume is simply additive. If, however, a very soft variety was mixed with a variety giving firm texture a very high improvement upon simple additiveness could be achieved. Diagram No. 3 in Fig. 7 shows that with the addition of as much as 25–50% Jubilejnaja 50 the loaf volume reached the size (largest cross section) of the loaf baked purely from Jubilejnaja 50.

The results permit the conclusion that in optimization of baking quality the rheological properties of gluten play a very important role. Of soft wheats of sufficiently high raw protein content is possible to bake good bread if an additive component of synergistic dough properties can be found. This is dependent largely beside the quantity of protein on its quality as well.

*

The author is highly indebted to Prof. Dr. H. BOLLING, director (Bundesforschungsanstalt für Getreide- und Kartoffelverarbeitung, Institut für Müllereitechnologie, Detmold, FRG) for support given by putting at her disposal his earlier results as control values.

Literature

BOLLING, H. (1977): Herstellung von Weizenmischungen in der Mühle. *Getreide, Mehl Brot, 10*, 261–265.

BOLLING, H. (1980): Zur Optimierung der Backeigenschaften von Weizenmischungen unter besondere Berücksichtigung spezifischer Rohstoffeigenschaften. *Getreide, Mehl Brot, 12*, 310–314.

BOLLING, H. (1983): Der Kombinationseffect spezifischer Rohstoffeigenschaften von Weizen im Minblick auf Verarbeitung und Sortenwertprüfung. *Allgemeiner Mühlen-Markt, 84*, 20–24.

GRAF, D. O. (1984): Getreideannahme, Vermahlung und Qualitätskontrolle. *Allgemeiner Mühlen-Markt, 85*, 215–217.

HORVÁTH-ALMÁSSY, K. (1986): Szedimentációs tesztek alkalmazhatóságának vizsgálata lisztkeverékek minősítésére. (Applicability of sedimentation tests for the qualification of flour mixtures.) *Sütőipar, 33*, 45–47.

KARÁCSONYI, L. (1970): *Gabona, liszt, sütő és tésztaipari vizsgálati módszerek.* (Analytical methods of cereals, flour and the baking and pastry industries.) Mezőgazdasági Könyvkiadó, Budapest, pp. 65–76; 105–120.

PALLAGI-BÁNKFALVI, E. & MATUZ, J. (1984): Correlation analyses of the SDS test and valorigraph values of autumn wheat varieties. *Acta Alimentaria, 13*, 303–308.

Acta Alimentaria, Vol. 18 (1), pp. 31–35 (1989)

DETERMINATION OF WHEAT PROTEIN BY A NEW SPECTROPHOTOMETRIC METHOD

E. GÁBOR

College Faculty of Food Industry, University of Horticulture and Food Industry, H-6701 Szeged, Marx tér 7. Hungary

(Received: 30 December 1987; accepted: 6 June 1988)

A new spectrophotometric method was elaborated to determine the protein content of wheat.

The principle of the method is based on the light absorption of the protein solution, which is proportional to the protein quantity present.

The examined material was digested with formic acid at 100 ± 1 °C for 60 minutes. After cooling, ethanol and methylene chloride were added to the reaction mixture. The spectrophotometric solution was made from this suspension by adding formic acid to it. The non-dissolved bran residues were filtered off before measurement of the optical density of the solution.

Calibration measurements were carried out by means of the Kjeldahl method for the calculation of protein content.

Mathematical evaluation demonstrated the good applicability of the spectrophotometric method.

Keywords: protein content, spectrophotometry, wheat, whole meal

The protein content is one of the most important factors for wheat qualification. The methods most often used for its determination are based on the Kjeldahl technique, which is modified in several ways to shorten the time of analysis and simplify the procedure.

The method we have elaborated for protein content determination by UV-spectrophotometry is based on the characteristic light absorption of the protein solution in the range of 300–250 nm, caused by the aromatic amino acids present as protein constituents in the system (SCHORMÜLLER, 1965).

The most important part of the method is the solubilization of the protein without harmful degradation of the sample.

A chemical digestion generally has to be carried out, sometimes in connection with some physical treatment, depending on the chemical constitution and structure of the examined material (NAKAI & LE, 1970; TOMA & NAKAI, 1971; GÁBOR, 1983; 1986; 1987).

In the case of wheat protein determination, some bran residues may be present in the system, which must be filtered off before spectrophotometry.

Total solubilization of the protein means that a certain heterogenity in the particle size of the well-ground material does not influence the accuracy of the method. Our measurements were carried out with whole meal.

1. Materials and methods

1.1. Preparation of solution

A sample of 0.4 g was weighed with 0.1 mg accuracy on a small glass sheet and placed in the reaction flask (Fig. 1), and 10.00 cm^3 of formic acid was added to it. The reaction flask was covered with the upper section and put in a waterbath of 100 $\pm$ 1 °C for 60 minutes. The tap in the upper part had to be

Fig. 1. Special reaction flask for sample digestion

closed after the reaction mixture had warmed up to the highest temperature, taking about 20–30 seconds. After heat treatment, the reaction mixture was cooled under tap water. Five cm^3 of ethanol and 5 cm^3 of methylene chloride were added next to the flask via its upper part. The tap was closed and the two parts of the flask held together by hand, the reaction mixture was vigorously shaken to achieve homogenization. This thin suspension was used to prepare the solution for spectrophotometry by diluting 0.50 cm^3 of it with 9.50 cm^3 of formic acid in a glass-stoppered tube. The bran residues were filtered off on a medium-pore filter. The clear solution produced in this way was suitable for spectrophotometric measurement.

The spectrophotometric blank solution was made in the same way, but did not contain the sample to be examined.

The optical density of the protein solution was measured at the absorption maximum, which was established from its spectrum. In our case it was at 281 nm.

1.2. Calibration measurements

Three samples with different protein contents were made from the material to be examined by mixing it with wheat starch in different proportions.

Table 1

Calibration measurements on whole meal (W M)

W M (g)	Protein content of W M (by Kjeldahl) in 20.00 cm³ suspension[a] (mg)	Protein content of 10.00 cm³ of spectrophotometric solution[a] (mg)	Optical density[a]
0.3000	36.51	0.910	0.512
0.4000	48.68	1.217	0.719
0.5000	60.85	1.520	0.866

[a] Average data of five parallels

The protein contents of the samples were analyzed both by the Kjeldahl technique as the basic method and by spectrophotometry. The data obtained are given in Table 1.

The regression equation was calculated as:

$$Y = a + b X \tag{1}$$

where Y = the optical density of the solution,

X = the protein content (mg) of the solution, calculated from the Kjeldahl data,

a, b = constant (in our case a = 0.0028; b = 0.581).

The correlation coefficient (r) was found to be 0.997, which means a good agreement between the two methods.

Only one calibration need to be carried out in the case of identical or similar samples.

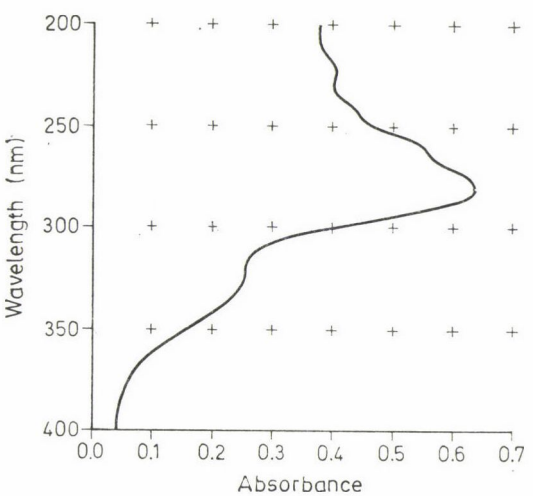


Fig. 2. Spectra of wheat protein solution (PU SP 8–100 UV spectrophotometer)

1.3. Calculation of percentage protein content of sample

The protein content of the spectrophotometric solution can be expressed from Eq. (1):

$$X = \frac{Y - a}{b} \tag{2}$$

Considering the known dilutions and sample weight (W, g), the following equation can be used:

$$\text{Protein content } (\%) = \frac{4X}{W}; \tag{3}$$

2. Results

The accuracy of the method was evaluated mathematically. Measurements (15 parallels) were carried out both with the Kjeldahl and spectrophotometric methods. Data are given in Table 2.

t-test

$$t_{calc} = 0.19;\ t_{table} = 1.76\ (P = 10\%)$$

Table 2

Measured and calculated protein contents of spectrophotometric solution

X_{meas} (mg)	Y (mg)	X_{calc} (mg)
1.233	0.718	1.231
1.239	0.722	1.239
1.220	0.724	1.241
1.219	0.710	1.217
1.205	0.702	1.203
1.219	0.705	1.209
1.205	0.701	1.202
1.185	0.707	1.212
1.185	0.698	1.196
1.203	0.710	1.217
1.193	0.688	1.179
1.212	0.692	1.184
1.202	0.710	1.217
1.213	0.688	1.179
1.191	0.710	1.217
1.202	0.705	1.209

X_{meas} = protein measured
Y = optical density
X_{calc} = protein calculated

This means that there is no significant difference between the average values of the two methods at 90% probability level.

F-test

$$F_{calc} = 1.23;\ F_{table} = 5.81\ (P = 10\%)$$

There is no significant difference between the variance values.

3. Conclusions

The mathematical evaluation proves the high accuracy of the spectrophotometric method. Rapidity and simplicity are additional advantages. It can be used both for raw material qualification and in research work.

The protein contents of other cereals and their products can likewise be determined by this method.

Literature

GÁBOR, E. (1983): Bestimmung des Eiweissgehalts von Fleisch und Fleischwaren mittels Spektrophotometrie im ultravioletten Bereich. *Fleisch, 37*, 194–196.

GÁBOR, E. (1986): Applicability and advantage of spectrophotometry in food quality control. *1st European Seminar of the EOQC Food Section, Budapest, Hungary.* Proceedings, pp. 180–191.

GÁBOR, E. (1987): Combined digesting method and spectrophotometry for complex food analyses. *2nd European Seminar of the EOQC Food Section, Zurich, Switzerland*, Seminar Working Document, pp. 27–37.

NAKAI, S. & LE, A. C. (1970): Spectrophotometric determination of protein and fat in milk simultaneously. *J. Dairy Sci., 53*, 276–278.

SCHORMÜLLER, J. (1965): *Die Bestandteile der Lebensmittel.* Springer Verlag, Berlin, p. 217.

TOMA, S. I. & NAKAI, S. (1971): Ultraviolet spectrophotometric determination of protein in some food products. *J. Fd Sci., 36*, 507–508.

Acta Alimentaria, Vol. 18 (1), pp. 37–44 (1989)

ESTIMATION OF LYSINE IN PIG MIXED FEEDS BY NEAR INFRARED REFLECTANCE ANALYSIS

K. Pazourek

Central Checking and Testing Institute of Agriculture,
Sokolovská 1. CS-186 00 Praha 8. Czechoslovakia

(Received: 13 January 1988; accepted: 14 June 1988)

Near infrared reflectance analysis (NIRA) is used for estimation of macro nutrients in mixed feeds in numbers of laboratories.

Classical lysine determination was performed in samples of two mostly produced categories of Czechoslovak pig feedmixes. Fifty-one samples of first category and 30 samples selected from a set of 55 samples of second category of pig mixed feeds by the "PICKS" programme were used for calibration of InfraAlyzer 400.

Lysine contents were in the range of 0.61–0.91 mass % in the first, and 0.41–0.72 mass % in the second category of mixed feeds, respectively. Values of standard error of calibration (SEC) were 0.029 mass % in the first, and 0.039 mass % in the second category of feed-mixes, respectively. Values of standard error of prediction (SEP) were 0.050 mass % in the first, and 0.047 mass % in the second category of pig mixed feeds, respectively. Reproducibility of NIRA is comparable with that of the classical method. Possibility of NIRA to predict constituents of low quantity and narrow range of occurrence, is discussed.

Keywords: Near infrared reflectance analysis, pig feed-mixes, lysine prediction, "PICKS" sample selection programme

Near infrared reflectance analysis (NIRA) is commonly used for prediction of various constituents of foods and fodders (Polesello & Giangiacomo, 1983). Experiences with NIRA application in protein prediction in mixed feeds were published by Williams and co-workers (1978) and Williams and Starkey (1980). They also examined the influence of some ingredients on the results of analysis of feed mixes. Chen and co-workers (1987) applied NIRA in moisture, protein and fibre prediction in five categories of pig mixed feeds produced in Taiwan. Fair results of application of NIRA in moisture, protein, fat, ash and fibre prediction in three categories of Czechoslovak pig feedmixes were obtained by Pazourek and Černý (1988).

NIRA was also used for estimation of amino acids. Rubenthaler and Bruinsma (1978) predicted lysine in cereals. Fourteen amino acids were predicted by Williams and co-workers (1984) in cereals and 16 amino acids by McGuire (1986) in distillers dried grain. Methionine in peas was estimated by Williams and co-workers (1985). All mentioned authors analyzed products where amino acids are bound in protein. L-cysteine mixed with other ingredients into wheat starch, making together a bread improving concentrate, was estimated by Osborne (1983). In the experiments here presented the lysine content

of pig mixed feed is increased by lysine hydrochloride contained in vitamin and mineral supplement added as one of the ingredients to the mixed feed for improving its nutritive value.

1. Materials and methods

1.1. *Preparation of the samples*

Samples of two mostly produced categories of pig mixed feeds were obtained from various feed mills of Zemedelské Zásobováni Co. in Bohemia during a 6 months period in 1986, 1987. These feed-mixes were classified into two categories according to composition (PAZOUREK and ČERNÝ (1988)). The first category contained feed-mixes for pigs weighing 17–65 kg and the second category contained feed-mixes for pigs weighing more than 65 kg. Thus the collected set of samples represents well the analyzed products in variability of ingredients content and range of lysine content.

Reference lysine determinations were performed on amino acid analyzer T 339 (Mikrotechna, Czechoslovakia) after hydrolysis of samples in sealed ampoules under nitrogen with hydrochloric acid (6 mol conc. HCl per dm^3) during 23 hours at 110 °C in drying oven. All analyses were performed in two replicates.

1.2. *NIR spectrometer*

InfraAlyzer 400 R connected with the computer HP-85 B (Technicon G.m.b.H., Vienna) was used for performing NIR analyses. Samples for NIRA were ground on a VM-7 mill (Zempo, Praha) and sieved through a screen of 1 mm mesh.

InfraAlyzer 400 measures light reflected from surface of the sample as reflectance $\left(\log \frac{1}{R}\right)$ at 19 wavelengths (filters). Results of analysis is reported after calculation according to regression formula:

$$\text{lysine}\,(\%) = F_0 + F_2 \log \frac{1}{R_2} + \ldots F_n + \log \frac{1}{R_n}$$

where

lysine (%) : lysine content in mass %
F_0, F_n : calibration constants (F values) for lysine predicting
R_i: reflectance value measured at i-th wavelength (filter)

1.3. *Calibration methods*

Utilization of a high number of wavelengths for any constituent prediction brings noise into measurements due to the intercorrelation between the filters. Therefore the programme "All best combination search" is used for selection of optimum combination of filters. This programme provides a possibility to perform such selection according to the value of the correlation coefficient and regression F-value (indicates how well the calibration line is predicting the data) after regression of 171 possible pairs, 969 triplets, etc. of filters. According to our experience published previously (PAZOUREK & ČERNÝ, 1988), sets of 6–9 filters are convenient for mixed feed analysis. This number of filters is probably determined by the composition of the analyzed product and corresponds to some results obtained in forage analysis (SHENK et al., 1978). The programme mentioned above was used for selection of filters for analysis of samples of both categories of mixed feeds.

Fifty-one samples were used for calibration of the instrument for the analysis of the first category of mixed feeds. A set of 55 samples of second category of feed-mixes was reduced into a set of 30 samples using the procedure known as "PICKS" programme. This procedure is based on a sample selection algorithm published by HONIGS and co-workers (1985). Near-infrared absorbance spectra are screened into a matrix which can be represented by a series of independent variables (i. e. spectral changes caused by changes of analyte concentration, interferences, instrument drift, detector noise, etc.) and their associated vectors. Sample selection is aimed to find a subset of spectra which contain all of the independent variables. This subset of samples is found after multiple application of the following procedure:

— Putting spectra of samples into a matrix — see example:

$$\log \frac{1}{R_{1,1}} + \log \frac{1}{R_{1,2}} + \ldots + \log \frac{1}{R_{1,20}}$$

$$\ldots$$

$$\log \frac{1}{R_{n,1}} + \log \frac{1}{R_{n,2}} + \ldots + \log \frac{1}{R_{n,20}}$$

— Finding of highest absolute value of $\log \frac{1}{R_{i,i}}$.

— Interchanging of matrix columns to bring this value into the first column.

— Subtracting selected fraction of first row from each of remaining spectra so that absorbance at the first wavelength is reduced to zero.

— Deleting the first row and first column of matrix and returning to step *a*).

Both calibration programmes were obtained from Technicon Company.

For comparison of reference results with predictions made by NIRA the values of Standard Error of Calibration (SEC) or Standard Error of Prediction (SEP) were calculated:

$$SEC = \sqrt{\frac{\Sigma(d_i - \bar{d})^2}{n - k - 1}}$$

$$SEP = \sqrt{\frac{\Sigma(d_i - \bar{d})^2}{n - 1}}$$

where

d_i: difference between the result of lysine determination by the classical method and its calculation by regression formula,
$\bar{d}$: average value of d_i,
n : number of samples in calibration (prediction) set,
k : number of wavelength used in calibration regression formula.

For comparison of reproducibility of reference and predicted results the values of Pooled Standard Deviation of classical (SD_{LAB}) and NIRA method (SD_{NIRA}) were used:

$$SD_{LAB(NIRA)} \sqrt{\frac{\Sigma d_i^2}{2n}}$$

where

d_i: difference between results of 2 parallel determinations of lysine by the classical method or NIRA,
n : number of analyzed samples.

Values of Regression F-ratio were used for evaluation, how close the predicted results are to the calibration line:

$$F = \frac{r^2(n - k - 1)}{(1 - r^2)k}$$

where

r: correlation coefficient,
n: number of calibration samples,
k: number of wavelength used in calibration regression formula.

The higher the F-ratio is, the closer the results are to the calibration line.

2. Results

Table 1 summarizes results of calibration of InfraAlyzer 400. It is evident that the range of lysine is narrower than that of the macro-nutrients as protein, fat, etc.

Table 1

Results of calibration of InfraAlyzer 400

	Mixed feeds for pigs weighing 17–65 kg	Mixed feeds for pigs weighing more than 65 kg
n	51	30
λ	2336, 2270, 2180, 2100, 1940, 1734, 1680	2208, 2139, 2180
r	0.951	0.912
SEC	0.029	0.039
Regression F-ratio	51.51	39.65
Range (mass %)	0.56—0.95	0.48—0.92
Average (mass %)	0.79	0.64

n: number of samples
λ: wavelengths (nm)
r: correlation coefficient
SEC: standard error of calibration

The set of 7 wavelengths used for lysine prediction in the first category of feed-mixes is empirical, but the set of 3 wavelengths used for analysis of the second category of feed-mixes can contain specific wavelengths for lysine estimation in this product. Values of regression F-ratio are at approximately equal level in both categories of mixed feeds. McGUIRE (1986) achieved a higher level of regression F-ratio predicting lysine in distillers dried grain. The decreased value of this quantity in lysine estimation in pig mixed feeds is probably caused by the homogeneity of the analyzed product and the repro-

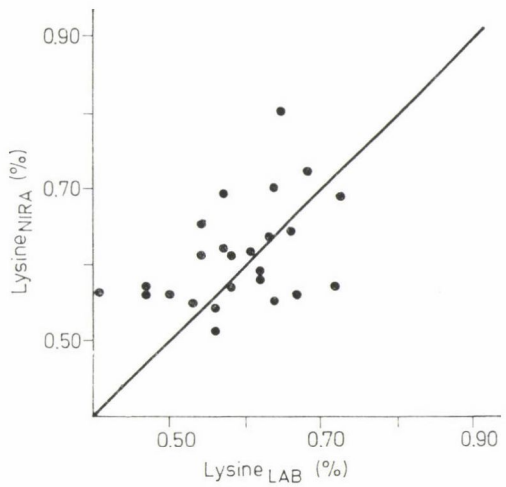


Fig. 1. Relationship between lysine content determined by amino acid analyzer and predicted by NIRA in mixed feeds for pigs weighing 17–65 kg

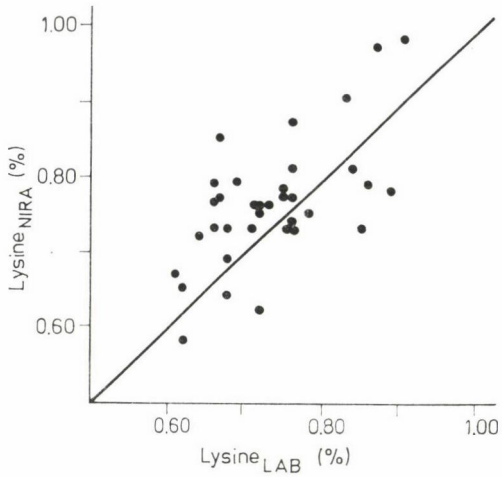


Fig. 2. Relationship between lysine content determined by amino acid analyzer and predicted by NIRA in mixed feeds for pigs weighing more than 65 kg

ducibility of the classical estimation of lysine in this type of product. This phenomenon should be subject to further study as the value of regression F-ratio influences the quality of predicted results.

Results of analysis of "unknown" samples are presented in Figures 1 and 2 and summarized in Table 2.

The relative error of lysine estimation in pig mixed feeds is higher than for the estimation of macro-nutrients by NIRA. Similar experiences were published by STARR and co-workers (1985) for NIRA prediction of glucosinolates in rape-seed. The problems mentioned seem to be common for NIRA application

Table 2

Results of analysis of "unknown" samples

	Mixed feeds for pigs weighing 17–65 kg	Mixed feeds for pigs weighing more than 65 kg
n	35	25
r	0.974	0.979
SEP	0.050	0.047
SD_{NIRA}	0.030	0.025
SD_{LAB}	0.021	0.025

n: number of samples
r: correlation coefficient
SEP: standard error of prediction
SD_{NIRA}: standard deviation of NIRA results
SD_{LAB}: standard deviation of laboratory results

in prediction of constituents of low quantity and close range in the analyzed product.

Values of SEP are approximately 1.3 times higher than those of SEC. Distance of 95% of the predicted results from reference ones is in the range of triple SEP (not double) as can be seen in Figures 1 and 2.

Pig mixed feeds contain lysine in two forms: bound in various proteins and added as lysine hydrochloride. This fact can also affect the reproducibility of lysine estimation by both methods. This is probably the reason why robust calibration can be hardly obtained in such cases.

Nevertheless the reproducibility of NIRA (measured by the values of SD in Table 2) is only slightly lower than the reproducibility of the classical method.

It is evident that the "PICKS" programme can be used for the selection of the set of calibration samples. Calibrations carried out without or with utilization of this programme gave comparable results in "unknown" sample set analysis. So the application of this programme is economical in cases where classical estimation of calibrated constituents is expensive and time consuming.

3. Conclusion

NIRA was used for lysine estimation in two categories of pig mixed feeds. According to the author's oppinion the accuracy of NIRA is in the described case satisfactory for screening of a high number of samples or for technology requirements.

Literature

CHEN, S.-L. Y, HSU, A. & LEE, M. L. (1987): Application of near infrared reflectance spectroscopy to compositional analysis of commercial pig feed mixes. *J. Ass. off. Analyt. Chem., 70,* 420–423.

HONIGS, D. E., HIEFTJE, G. M., MARK, H. L. & HIRSCHFELD, T. B. (1985): Uniquesample selection via near infrared spectral subtraction. *Analyt. Chem., 57,* 2299–2303.

MCGUIRE, C. F. (1986): Quality evaluation of distillers dried grain by near-infrared analysis. *Cereal Chem., 63,* 155–159.

OSBORNE, B. G. (1983): Measurement of levels of bread improvers in concentrates by means of near infrared reflectance spectroscopy. *J. Sci. Fd Agric., 34,* 1297–1301.

PAZOUREK, K. & ČERNÝ, M. (1988): Application of near infrared reflectance analysis in selected pig feed-mixes analysis. *Acta Alimentaria, 17,* 113–125.

POLESELLO, A. & GIANGIACOMO, R. (1983): Application of near infrared spectrophotometry to the nondestructive analysis of foods: a review of experimental results. *CRC Critical Rev. Fd Sci. Agric., 18,* 203–230.

RUBENTHALER, G. L. & BRUINSMA, B. L. (1978): Lysine estimation in cereals by near infrared reflectance. *Crop Sci., 18,* 1039–1042.

SHENK, J. S., WESTERHOUS, M. O. & HOOVER, M. R. (1978): Infrared reflectance analysis of forages. *International Grain and Forage Harvesting Conference Proceedings.* American Society of Agricultural Engineers. St. Joseph, pp. 242–244, 252.

STARR, C., SUTTLE, J., MORGAN, A. G. & SMITH, D. B. (1985): The comparison of sample preparation and calibration techniques for the estimation of nitrogen, oil and

glucosinolate content of rapeseed by near infrared spectroscopy. *J. agric. Sci. Camb.*, *104*, 317–323.

WILLIAMS, P. C., STEVENSON, S. G., STARKEY, P. M. & HAWTIN, G. C. (1978): The application of near infrared reflectance spectroscopy to protein testing in pulse breeding programmes. *J. Sci. Fd Agric.*, *29*, 285–292.

WILLIAMS, P. C. & STARKEY, P. M. (1980): Influence of feed ingredients upon the prediction of protein in animal feed mixes by near infrared reflectance spectroscopy. *J. Sci. Fd Agric.*, *31*, 1201–1213.

WILLIAMS, P. C., PRESTON, K. R., NORRIS, K. H. & STARKEY, P. M. (1984): Determination of amino acids in wheat and barley by near infrared reflectance spectroscopy. *J. Fd Sci.*, *49*, 17–20.

WILLIAMS, P. C., MACKENZIE, S. L. & STARKEY, P. M. (1985): Determination of methionine in peas by near infrared reflectance spectroscopy (NIRS). *J. Agric. Fd Chem.*, *33*, 811–815.

Acta Alimentaria, Vol. 18 (1), pp. 45–52 (1989)

IRRADIATION DISINFESTATION AND BIOCHEMICAL QUALITY OF DRY NUTS

ABDUS SATTAR, M. JAN, A. AHMAD, M. WAHID and I. KHAN

Nuclear Institute for Food and Agriculture, Tarnab, Peshawar, Pakistan

(Received: 15 January 1988; accepted: 3 May 1988)

The effectiveness of radiation disinfestation of Pakistani nuts such as almond, groundnut, pine nut and walnut, was studied. Species of insects involved were determined. Concentration of potential nutrients, phytate, total phosphorus and iron contents were determined. *Tribolium castaneum* and *Sitotroga cerealella* infested walnut and pine nut while *Cadra cautella* and *Plodia interpunctella* affected almond and groundnut, respectively. Irradiation dose of 1.0 kGy completely checked infestation of these nuts. Sensory ratings decreased with advanced storage, and the samples treated at 0.50 kGy were rated higher than others. Peroxidation rate was higher in pine nut and walnut than almond and groundnut. These nuts widely varied in the contents of potential nutrients such as protein, lipids, mineral matter, fibre and carbohydrates and exhibited wide range values of energy (2533–3102 kJ per 100 g), phytate (274.8–328.5 mg per 100 g), total phosphorus (268.5–604.0 mg per 100 g) and iron (6.6–11.6 mg per 100 g).

Keywords: irradiation, disinfestation, potential nutrients, peroxidation, phytate

Historically, dry nuts played a prominent role in the feeding of man and beasts in many civilizations and in different parts of the globe. They are considered a major source of income and foreign exchange in many countries and are utilized throughout the year, especially during space and other military missions. Nuts are a concentrated food and are widely consumed by people in the northern area of Pakistan.

Spoilage of dry nuts as a result of insect infestation and oxidative changes is a serious problem, particularly under humid tropical conditions such as exist in Pakistan. The use of gamma radiation for disinfestation of insects in fresh and dry fruit and nuts has been attempted by some specialists (MOY et al. 1983; ROVETTI, 1975; BROWER & TILTON, 1970; GONZALES, 1975). However, conflicting results have been reported as to the insects involved and optimum radiation dose needed for disinfestation. Oxidative deterioration of dry nuts under tropical conditions has been little studied. Although nuts have been reported to contain generally higher levels of phytate (MAGA, 1982), evaluation of Pakistani dry nuts for this antinutrient has not been attempted.

The objective of this study was to evaluate the quality of dry nuts with regard to insect infestation, potential nutrients, phytate and peroxidation.

1. Materials and methods

Fresh samples of dry nuts such as almond, groundnut, pine nut and walnut were obtained from the local dry-fruit market at Peshawar. They were shelled carefully enough to avoid breakage of kernels. The kernels in each case were kept in clear polyethylene pouches at ambient temperature (28–40 °C) for subsequent radiation treatment and chemical analysis.

Samples after packaging in polyethylene pouches were irradiated at dose levels of 0.25, 0.50, 0.75 and 1.00 kGy in a Cobalt-60 gamma irradiator (Issledovatel, USSR) having a dose rate of 9.6 kGy per hour. Percentage damage of dry nuts by the insects was determined on the basis of damaged kernels by weight according to COGBURN (1977). The samples were examined during storage for the nature of insect infestation following the methods of BLAND (1978). Sensory evaluations were conducted by hedonic ratings on a 9-point scale (LARMOND, 1977).

The samples were analysed for moisture, total mineral matter (ash), lipids (fat), protein, fibre, carbohydrates, energy value, iron, total phosphorus and phytic acid. Proximate analysis was performed in duplicate in accordance with AOAC (1975). Moisture was determined in a drying oven at 105 °C. Determination of fat was carried out by Soxhlet extraction using petroleum ether (b. p. 40–60 °C) in a Soxtec system HT (Tecator) and protein N(%) × 6.25) by micro-Kjeldahl method. Ash was determined by heating at 550 °C and fibre by digestion with acid and alkali using Fibertec system M (Tecator). The food energy was calculated from the proximate analysis data by multiplying the fat by 37.7, and protein and carbohydrates by 16.7 kJ g^{-1}. The wet ashing procedure of SATTAR and CHAUDRY (1978) was used for iron and phosphorus. Iron was determined by using potassium thiocyanate and total phosphorus using ammonium molybdate and ammonium vanadate by spectrophotometry (AOAC 1975). Extraction, precipitation and determination of phytate phosphorus were carried out according to the method of WHEELER and FERREL (1971); assuming an iron phosphorus ratio of 4 : 6 in the ferric phytate. Peroxidation was followed according to LEA (1952).

2. Results and discussion

The influence of gamma irradiation and storage on the extent and nature of insect infestation in dry nuts is presented in Table 1. The data revealed that only higher dose (1.0 kGy) was effective to completely check infestation in almond, groundnut, pine nut and walnut. Low doses (0.25, 0.50 and 0.75 kGy) decreased insect infestation differentially depending on the dose level and the type of dry nut.

Table 1

Effect of irradiation and storage on the insect infestation in dry nuts

Dry nuts	Storage period (month)	Infestation (%) Irradiation dose (kGy) 0	0.25	0.50	0.75	1.00	Insects
Almond	2	5	3	1	0	0	*Cadra cautella*
	4	28	15	5	0	0	
	6	45	40	30	0	0	
Groundnut	2	20	7	4	0	0	*Plodia interpunctella*
	4	47	18	7	0	0	
	6	65	50	40	10	0	
Pine nut	2	16	13	5	0	0 i/	*Tribolium castaneum*
	4	58	40	13	0	0 ii/	*Sitotroga cerealella*
	6	100	80	60	26	0	
Walnut	2	15	10	2	0	0 i/	*Tribolium castaneum*
	4	60	40	17	0	0 ii/	*Sitotroga cerealella*
	6	100	100	100	45	0	

Initial infestation = zero

Initially the infestation was zero, however, it increased with advancing storage upto a period of 6 months. The rate of infestation was generally higher in walnut and pine nut than almond and groundnut. The unirradiated samples of walnut and pine nut were found completely infested by insects after 6 months storage whereas infestation was comparatively lower in almond and groundnut. Identification of the infesting insects indicated that walnut and pine nut were attacked by *Tribolium castaneum* and *Sitotroga cerealella*, almond by *Cadra cautella* and groundnut by *Plodia interpunctella*. It appears that the relatively soft texture and high moisture of walnut and pine nut kernels might be a reason of higher infestation as compared to kernels of almond and groundnut which had hard texture and seed coats and low moisture levels. The implication of these findings is that the relationship between the effective dose and the degree of moisture level and texture of nuts, is important. In the literature, there are conflicting results as to the optimum dose of gamma irradiation in controlling insects in dry fruits and nuts. GONZALES (1975) reported that a dose of 0.1 kGy was sufficient to suppress insect infestation in stored products. However, ROVETTI (1975) and BROWER and TILTON (1970; 1972) recommended a dose range of 0.3–0.4 kGy for preventing insect development and feeding damage in dry fruits and nuts. The influence of irradiation doses and storage on the sensory quality of these nuts was monitored and the results of overall acceptability for colour, texture and flavour are shown in

Table 2

Effect of irradiation and storage on the sensory quality of dry nuts

Dry nuts	Storage (month)	Irradiation dose (kGy)					Mean value of sensory test	CV (%)
		0	0.25	0.50	0.75	1.00		
Almond	2	8.0	8.6	9.0	8.6	8.0	8.44	5.14
	4	7.0	7.0	7.5	7.2	7.0	7.14	3.07
	6	5.0	6.0	6.5	6.6	6.0	6.02	10.53
Groundnut	2	7.0	7.5	7.8	7.7	7.4	7.48	4.16
	4	6.0	7.6	7.5	7.5	7.3	7.18	9.31
	6	4.7	5.0	6.0	6.2	5.4	5.46	11.70
Pine nut	2	7.0	7.3	7.5	8.0	7.2	7.40	5.15
	4	5.5	6.8	7.6	6.7	6.6	6.64	11.30
	6	3.0	4.6	5.5	5.5	5.1	4.74	21.96
Walnut	2	7.0	7.8	8.3	8.1	8.0	7.84	6.42
	4	5.0	6.4	6.5	6.5	6.2	6.12	10.42
	6	3.0	4.0	5.0	5.1	4.5	4.32	19.87
Mean value		5.68	6.55	7.06	6.98	6.56		
CV (%)		28.40	21.41	16.63	15.31	17.31		

CV: coefficient of variation

Table 2. It was observed that the storage period had a marked damaging effect on the overall acceptability scores. The decrease in mean scores was higher in the unirradiated dry nuts and those irradiated with $\geq$0.75 kGy than other treatments tested. Comparatively samples treated at 0.50 kGy were rated higher in each case. Lower ratings for unirradiated controls were probably due to greater insect infestation while for 0.75 and 1.00 kGy treated samples due to higher peroxidation. Since good to excellent correlations have been reported between sensory ratings and oxidized fats and oils (SATTAR et al., 1976) the influence of irradiation doses on the peroxide value of dry nut oils was not studied.

Peroxidation rate of dry nuts under room conditions (28–40 °C), determined during a storage of 24 weeks, is shown in Fig. 1. It was observed that the rate of peroxidation was higher in walnut and pine nut than almond and groundnut. A similar trend of the insect infestation was observed in the unirradiated contents. There is little or no information available in the literature as to the development of oxidative rancidity storage under humid tropical conditions. KHAN and co-workers (1985) and WAHID and co-workers (1987) have shown the occurrence of greater losses in dry fruits due to insect infestation and other chemical changes such as discolouration and vitamin losses, during storage.

Regarding peroxide value, p-anisidine value and free fatty acid content, JAN and co-workers (1988) found no statistically significant differences between unirradiated and irradiated (0.5 kGy and 1.0 kGy, resp.) samples of walnuts neither directly after irradiation, nor during storage for 15 weeks. However regardless to irradiation lipid oxidation progressed during storage therefore

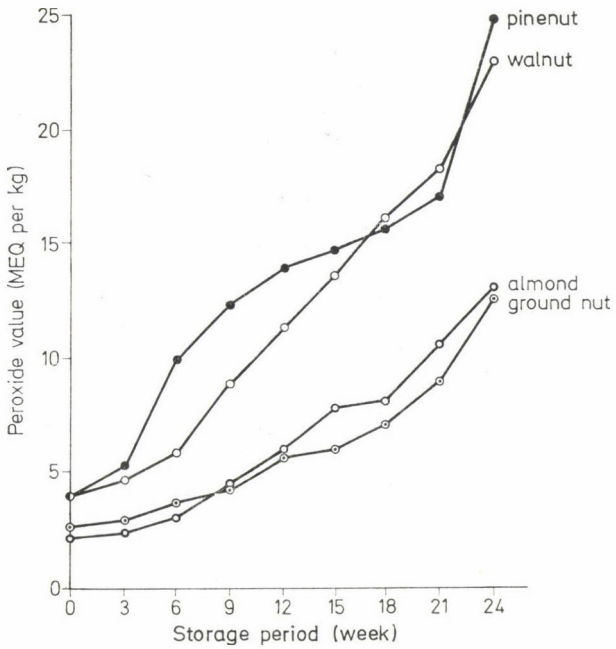


Fig. 1. Peroxidation of dry nuts during storage under tropical conditions

they suggested that shelled walnuts should be packed in tin or laminate with N_2 gas in order to prevent major deterioration of quality during storage at ambient temperature.

In view of the dietary importance of dry nuts for people living in northern hilly areas of Pakistan who are famous for longevity, these nuts were analysed for potential nutrients, Fe, P and an antinutrient phytic acid which can chelate iron and other essential metals thereby causing iron and other deficiencies among people of these areas. The results shown in Table 3 indicate that moisture percentage was slightly higher in pine nut and walnut than almond and groundnut. Nuts were found to be a best source of potential nutrients such as ash, protein, fat, fibre and carbohydrates as well as energy. Walnut contained the highest amount of fat (66.0%) followed by almond (48.5%), pine nut (46.5%) and groundnut (38.6%). Protein content was highest in groundnut (37.5%) intermediate in almond (26.2%) and pine nut (21.0%) and lowest in walnut (19.4%).

Table 3

Potential nutrients of dry nuts

Dry nuts	Moisture (%)	Mineral matter (%)	Protein (%)	Fat (%)	Fibre (%)	Carbohydrates (%)	Energy (kJ per 100 g)
Almond	4.0	1.8	26.2	48.5	2.9	17.9	2767
Groundnut	4.0	2.7	37.6	38.6	1.9	17.6	2533
Pine nut	5.5	2.4	21.0	46.5	0.8	26.8	2696
Walnut	5.5	1.0	19.4	66.0	1.6	11.1	3102
Mean value	4.75	1.97	26.02	49.90	1.80	18.35	2774.5
CV (%)	18.23	38.07	31.44	23.05	48.21	35.13	8.61

The values are the averages of 2 determinations

Table 4

Phytate, non-phytate, total phosphorus and iron content of dry nuts

Dry nuts	Phytate phosphorus (mg per 100 g)	Non-phytate phosphorus (mg per 100 g)	Total phosphorus (mg per 100 g)	Iron (mg per 100 g)
Almond	302.0	130.0	432.0	11.6
Groundnut	274.8	193.7	468.5	8.2
Pine nut	317.6	195.9	513.5	6.6
Walnut	328.5	275.5	604.0	8.4
Mean value	305.72	198.77	504.5	7.57
CV (%)	7.62	29.97	14.71	13.53

The values are the averages of 2 determinations

In order to make an estimate of the dispersion of the amount of individual nutrients in nuts, the coefficient of variation (CV) was measured. This revealed striking differences in the contents of potential nutrients among various nuts. Determination of CV is specially appropriate under conditions where there are extreme values or when it is desired to express variation as a percentage of the average around which the deviations are taken. Phytate, non-phytate phosphorus and iron contents of whole kernels are presented in Table 4.

The highest amounts of phytate was found in walnut (328.5 mg per 100 g), lowest in groundnut (274.6 mg per 100 g) and intermediate in almond (302.0 mg per 100 g) and pine nut (317.6 mg per 100 g). These values are higher than generally reported for cereals and legumes. This suggests that the intake of higher quantities of nuts could seriously affect bioavailability of essential mineral elements. Similar pattern of the concentration of total phosphorus in these nuts was observed. Iron content in the whole kernels indicated slightly varying amounts among these nuts and a range value of 6.6–11.6 mg per 100 g was observed.

The values for Fe seem to be generally low as compared to most vegetables, cereals and legumes. Further, being a concentrated food, dietary intake of nuts is definitely lower than other food materials. Prevalence of the iron deficiency among people of these northern areas might well be linked to higher levels of phytate and lower intake of dietary iron. Again, determination of CV revealed greater dispersion in the content of total phosphorus, intermediate in iron and lower in phytate phosphorus; obviously non-phytate phosphorus followed the pattern of total phosphorus. Indeed higher level of phytate in different nuts has been reported by MAGA (1982); however, there is no reported data available as to the phytate and other metal contents of Pakistani nuts.

It was concluded from these studies that these nuts although contained high amount of potential nutrients, they also have appreciable amount of phytate which can reduce bioavailability of essential metals. Peroxidation and insect infestation of these nuts is a matter of concern during storage under tropical conditions. Although insect disinfestation of these nuts can be achieved by irradiation treatment with 1.00 kGy, suitability of packaging materials for reducing development of rancidity needs investigation.

Literature

A.O.A.C. (1975): *Official methods of analysis.* 12th ed. Association of Official Analytical Chemists, Washington, D.C.

BLAND, R. C. (1978): *How to know the insects?* Wm. C. Brown Company Publishers Dubuque, Iowa.

BROWER, J. H. & TILTON, E. W. (1970): Insect disinfestation of peanuts by gamma radiation. *J. Georgia Ent., 50,* 199–203.

BROWER, J. H. & TILTON, E. W. (1972): Insect disinfestation of shelled pecans, almonds and walnuts by gamma radiation. *J. econ. Ent., 65,* 222–224.

COGBURN, R. R. (1977): Susceptibility of varieties of stored rough rice to losses caused by storage insects. *J. Stored. Prod. Res., 13,* 29–34.

GONZALES, Q. N. (1975): Radiation inactivation of insects. *Fd Irrad. Inf., 4,* 35–37.

JAN, M., LANGERAK, D. Is., WOLTERS, TH. G., FARKAS, J., KAMP, H. J. V. D. & MUUSE, B. G.:(1988):The effect of packaging and storage conditions on the keeping quality of walnuts treated with disinfestation doses of gamma rays. *Acta Alimentaria, 17,* 13–31.

KHAN, I., SATTAR, A., WAHID, M. & JAN, M. (1985): Radiation disinfestation of dry fruits. — in: *Proc. Intern. Conf. Radiation Disinfestation of Food and Agric. Products.* Honolulu, Hawaii, USA. 1983, pp. 207–213.

LARMOND, E. (1977): *Methods for sensory evaluation of food.* Canada Department of Agriculture, Publ. No. 1284. pp. 36–37.

LEA, C. H. (1952): Methods for determining peroxide in lipids. *J. Sci. Fd Agric., 3,* 586–594.

MAGA, J. A. (1982): Phytate, its chemistry, occurrence, food interactions, nutritional significance and methods of analysis. *J. agric. Fd Chem., 30,* 1–9.

MOY, J. H., KANASHIRO, K. Y., OHTA, A. T. & NAGAI, N. (1983): Radiation disinfestation of California stone fruits infested by Medfly-effectiveness and fruit quality. *J. agric. Fd Chem., 30,* 1–9.

ROVETTI, L. M. (1975): Radiation treatment of various commodities of Venezuela. *Fd Irrad. Inf. 4,* 61–62.

SATTAR, A. & CHAUDRY, M. A. (1978): Trace element eontents of food and their interrelationships with protein values in milled fractions of wheat and tritical. *Pakistan J. Biochem., 11,* 48–54.

SATTAR, A., DEMAN, J. M. & ALEXANDER, J. C. (1976): Light induced oxidation of edible oils and fats. *Lebensm. Wiss. Technol., 9,* 149–152.

WAHID, M., SATTAR, A. NEELOFAR, ATTA, S., KHAN, I. & EHLERMANN, D. A. E. (1978): Radiation disinfestation and quality of dried fruits. *Acta Alimentaria, 16,* 159–166.

WHEELER, E. L. & FERREL, R. E. (1971): A method for phytic acid determinations in wheat and wheat fractions. *Cereal Chem., 48,* 312–320.

Acta Alimentaria, Vol. 18 (1), pp. 53–63 (1989)

ISOLATION, PURIFICATION AND DETERMINATION OF LOW MOLECULAR WEIGHT PEPTIDES FROM BREAD DOUGH[a]

C. BENEDITO DE BARBER, J. A. PRIETO, C. COLLAR and S. BARBER

Laboratorio de Cereales, Instituto de Agroquímica y Tecnologia de Alimentos (CSIC), Jaime Roig, 11. 46010 Valencia, Spain

(Received: 27 January 1988; accepted: 26 June 1988)

Efficiency and suitability of different solvents, 1 mol l^{-1} acetic acid (i), distilled water (ii) and 70% ethanol (iii), for the extraction of low molecular weight peptide fraction from unfermented wheat flour dough are tested. Purification of dough soluble fraction (SN) was carried out following the sequence: ultrafiltration (i, ii) or absolute ethanol precipitation (iii), ion exchange chromatography on Dowex 50W–X2, molecular exclusion chromatography on Sephadex G–10 and ligand exchange chromatography on Cu^{2+}-Sephadex, for each solvent tested. Low molecular weight peptide content was estimated in each purified extract by analysing primary amino nitrogen (AN). Results obtained show that the methodology used is suitable for the isolation and purification of low molecular weight peptides being their estimated content, larger in the acetic acid extract, 32.6 mg per 100 g dough, d. b. (i), than in the other extracts: 24.0 mg per 100 g dough, d. b. (ii) and 26.9 mg per 100 g dough, d. b. (iii).

Keywords: amino acids, bread dough, ligand exchange chromatography, low molecular weight peptides purification, proteins and polypeptides electrophoresis

Peptides constitute a group of components whose role in breadmaking and in bread quality have hardly been considered to date. This is the case, in spite of the fact they are, according to the case, potential antioxidants, flavor enhancers, sweeteners or bitter principles (FUJIMAKI, 1980). Their changes during fermentation, the key step in breadmaking, remain unknown; nevertheless, this step offers several opportunities for the formation as well as the transformation and consumption of peptides. Breadmaking system (direct, sponge, sponge-dough) and fermentation (time, temperature and humidity) constitute additional factors associated with the trend and the extent of the probable changes. Despite the evident interest of low molecular weight peptides in bread dough fermentation, very little work has been done in this field. This is due in part to difficulties in the quantitative extraction of the peptide fraction and the subsequent peptide determination, since they are co-extracted with free amino acids, which implies an additional purification step.

[a] Paper presented at the II. World Congress of Food Technology, Barcelona, Spain, March 3–6, 1987

Solvents most widely used by different authors for the extraction of amino acids and/or peptides from flour, dough and bread have been: distilled water, 70% ethanol and acetic acid solutions (JONES & CARNEGIE, 1969; EL-DASH, 1971; EL-DASH & JOHNSON, 1970; FARIDI & JOHNSON, 1978; HORVAT et al., 1962; KRETOVICH & PONOMAREVA, 1961; LINKO & JOHNSON, 1963; MORIMOTO, 1966; PONOMAREVA et al., 1964). However, a study of their extractability by different solvents has been published only for the extraction of peptides from flour (JONES & CARNEGIE, 1969). Data referring to bread-dough have not been found. The scarcity of data published about the extraction behaviour and purification of low molecular weight peptides has led us to initiate, with the present study, a line of work designed to clarify the role of peptides in breadmaking being the immediate aims:

— to learn about the capacity and suitability of different solvents for the extraction of low molecular weight peptides from bread dough,

— to establish a purification sequence and

— to obtain preliminary data on the low molecular weight peptide content from unfermented bread dough.

1. Materials and methods

1.1. Material

Commercial wheat bread flour of $W = 110 \times 10^3$ ergs and $P/L = 0.71$ was choosen. Bread doughs were elaborated with commercial pressed yeast.

1.2. Methods

1.2.1. *Elaboration of bread doughs.* Bread doughs were formulated by adding to each 100 g flour, 56.5 g water, 2 g yeast and 1.8 g salt. Three hundred g flour samples were mixed in the mixer of a Brabender farinograph for 3 min at 90 r.p.m.

1.2.2. Extraction of nitrogen compounds. Nitrogen compounds were extracted twice from 200 g dough samples, m.b., by three different solvents: i) 1 mol l^{-1} acetic, ii) distilled water and iii) 70% ethanol, in the ratio of 200 : 225 (w/v). Extracts were centrifugated at 23 300 g for 20 min at 1–3 °C (i, ii) and at 5 000 r.p.m. for 50 min at 1–3 °C (iii). Supernatants were filtered through Whatman No. 1 paper. In addition, acetic acid soluble fraction was kept refrigerated for 3–4 h in order to allow proteins causing turbidity to precipitate; precipitate was removed by centrifugation at 23 300 g for 20 min at 1–3 °C and subsequent decanting of the supernatant. Finally, supernatants containing the soluble fraction (SN) were made up to 500 cm^3. A scheme of the different steps used for the extraction and purification of nitrogenous compounds is summarized in Fig. 1.

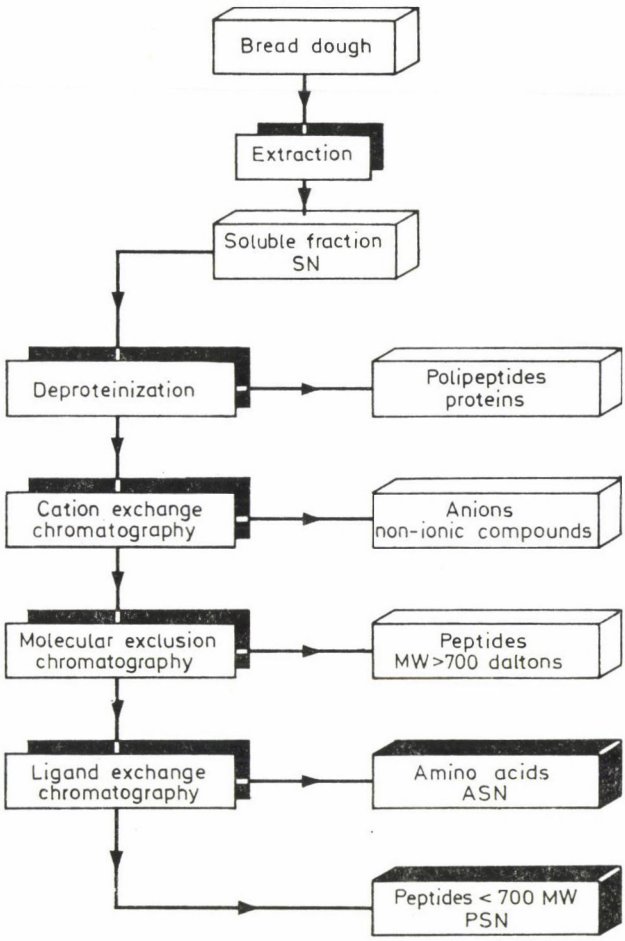


Fig. 1. Simplified scheme of the isolation and purification of soluble fraction (SN), purified soluble peptide fraction (PSN) and purified soluble amino acid fraction (ASN) from unfermented wheat bread doughs. The methodology performed is detailed in para. 1

1.2.3. Deproteinization. 1 mol l^{-1} acetic acid (SNi) and distilled water (SNii) soluble fractions from bread doughs were deproteinized by ultrafiltration through a cartridge, cut-off: 10 000 daltons. An aliquot of SN was ultrafiltered against distilled water, ratio SN : H_2O = 4 : 25, at 4 °C, flow rate: 200–250 cm^3h^{-1}. Seventy percent ethanol soluble fractions (SNiii) were deproteinized in two successive steps by absolute ethanol precipitation, ratio SN : EtOH = 1 : 2. Protein free extracts were obtained by centrifugation at 27 000 g for 30 min at 1–3 °C.

1.2.4. Ion exchange chromatography. Aliquots of the protein free extracts were acidified with glacial acetic acid to pH 2.0 and applied to a column (2×18 cm)

packed with a bed of Dowex 50W—X2 resin (50–100 mesh, K^+ form) previously equilibrated with 0.01 mol l^{-1} HCl. Anions and non-ionic compounds were eluted with 250 cm^3 of distilled water, whereas amino acids and peptides were eluted with 500 cm^3 of 4 mol l^{-1} NH_4OH. Respective eluates, from which the remaining ammonia was removed on a rotary evaporator, were redissolved in distilled water and centrifuged at 2 000 r.p.m. for 10 min, in order to remove insoluble material.

1.2.5. Molecular exclusion chromatography. A 5 cm^3 of each extract partially purified by cation-exchange chromatography was applied to a column (25 × 80 cm) containing a Sephadex G-10 bed. Samples were eluted with distilled water at a flow rate of 1 cm^3 min^{-1} and collected in fractions of 6 cm^3 each. Peptides excluded from the gel (MW > 700 daltons) were detected by polyacrylamide slab gel electrophoresis (HASHIMOTO et al., 1983) and by α-amino nitrogen of free amino acids (AAN) and primary amine nitrogen (AN) determinations before and after acid hydrolysis of an aliquot of each fraction.

1.2.6. Ligand exchange chromatography. Aliquots of each fraction collected from the Sephadex G-10 column, containing amino acids and low molecular weight peptides, were combined, freeze-dried and redissolved in 1 cm^3 of 50 mmol l^{-1}, pH 11 $Na_2B_4O_7$ buffer, and then applied to a column (bed: 1.5 × 11 cm) packed with Cu^{2+} Sephadex. Samples were eluted following the chromatographic conditions described by ROTHENBUHLER and co-workers (1979); Cu^{2+} was removed from the eluates by cation exchange chromatography on Chelex-100 resin.

1.2.7. Determination of total solids (TS), total nitrogen (TN), primary amine nitrogen (AN) and α-amine nitrogen of free amino acids (AAN) contents. TS content was determined after freeze-drying, TN by the Kjeldahl semimicro method (USP, 1975); AN, by the modified ninhydrin method (SPACKMAN et al., 1958), and AAN, by the original cadmium–ninhydrin method described by DOI and co-workers (1981).

1.2.8. Electrophoresis of proteins and low molecular weight polypeptides. Polyacrylamide (PA) slab gel electrophoresis using dissociating discontinuous buffer system (SDS-PAGE) was performed on 10% PA for separating proteins (LAEMMLI, 1970) and on a gradient from 10 to 18% PA for separating polypeptides (HASHIMOTO et al., 1983).

1.2.9. Acid hydrolysis. Acid hydrolysis was carried out in evacuated sealed tubes with 0.5 cm^3 6 mol l^{-1} HCl at 110 °C for 22 h. Hydrolysates were filtered and HCl removed in a freeze dryer. Dry residues were dissolved in distilled water, freeze-dried and redissolved in distilled water for analysis.

2. Results

2.1. Solvents

Suitability of the solvents tested for the extraction of the peptide fraction from dough was evaluated by TS, TN, AN and AAN determinations, and additionally, by characterization of the electrophoretic behaviour of proteins and low molecular weight peptides from the soluble fraction of dough (SN).

Results obtained for SN show that nitrogen components from dough undergo changes in extractability (AN, AAN) and in selectivity (AN and AAN vs TN and TS) depending on the solvent used for the extraction (Table 1). Low molecular weight nitrogen compounds are most exhaustively extracted by acetic acid. AN and AAN contents of the acetic acid extract are significantly (P = 95%) greater (by 13 and 12%, respectively) than those of the distilled water extract and (by 21% and 43%, respectively) than those of the ethanol extract. However, percentages of AN and AAN as a ratio to TN, which are significantly higher for the distilled water extract (6.83% and 3.81%, respectively) than for the acetic acid extract (5.10% and 2.81%) and for the ethanol extract (1.59% and 0.57%) show that the water soluble nitrogen fraction is richer in peptides and free amino acids. When AN and AAN contents are considered with respect to TS contents, differences among extracts are not important between water extract (0.23% and 0.13%, respectively) and acetic acid extract (0.21% and 0.12%), while they are significant when data are compared with the ethanol extract (0.12% and 0.04%).

Differences in AN and AAN among extracts indicate that differences in extractability of the protein fractions, are mainly responsible for the difference in TN. Parallel studies done with higher molecular weight fractions (proteins and polypeptides of SN) also showed differences due to the extractability of different solvents. Ten % polyacrylamide gel electrophoresis (LAEMMLI, 1970)

Table 1

Primary amino nitrogen (AN) and α-amino nitrogen of free amino acids (AAN) contents of 1 mol l^{-1} acetic acid (SNi), distilled water (SNii) and 70% ethanol (SNiii) soluble fractions from unfermented bread doughs

Fraction	n	AN ($g \times 10^3$ per 100 g dough, d. b.)				n	AAN** ($g \times 10^3$ per 100 g dough, d. b.)		
		x	±s	CL	VC (%)		x	±s	VC (%)
SNi	4	19.835	0.711	1.132	3.58	4	15.904	0.238	2.18
SNii	4	17.181	0.511	0.812	2.97	4	9.567	0.326	3.41
SNiii	4	15.635	0.581	0.446	3.71	4	5.586	0.246	4.40

** Statistically significant differences (P = 99%) among AAN mean values ($Se^2 = 0.0495$, $F = 620$, $SD(Q^{95}_{3,9}) = 0.439$

VC: variation coefficient

n: number of measurements

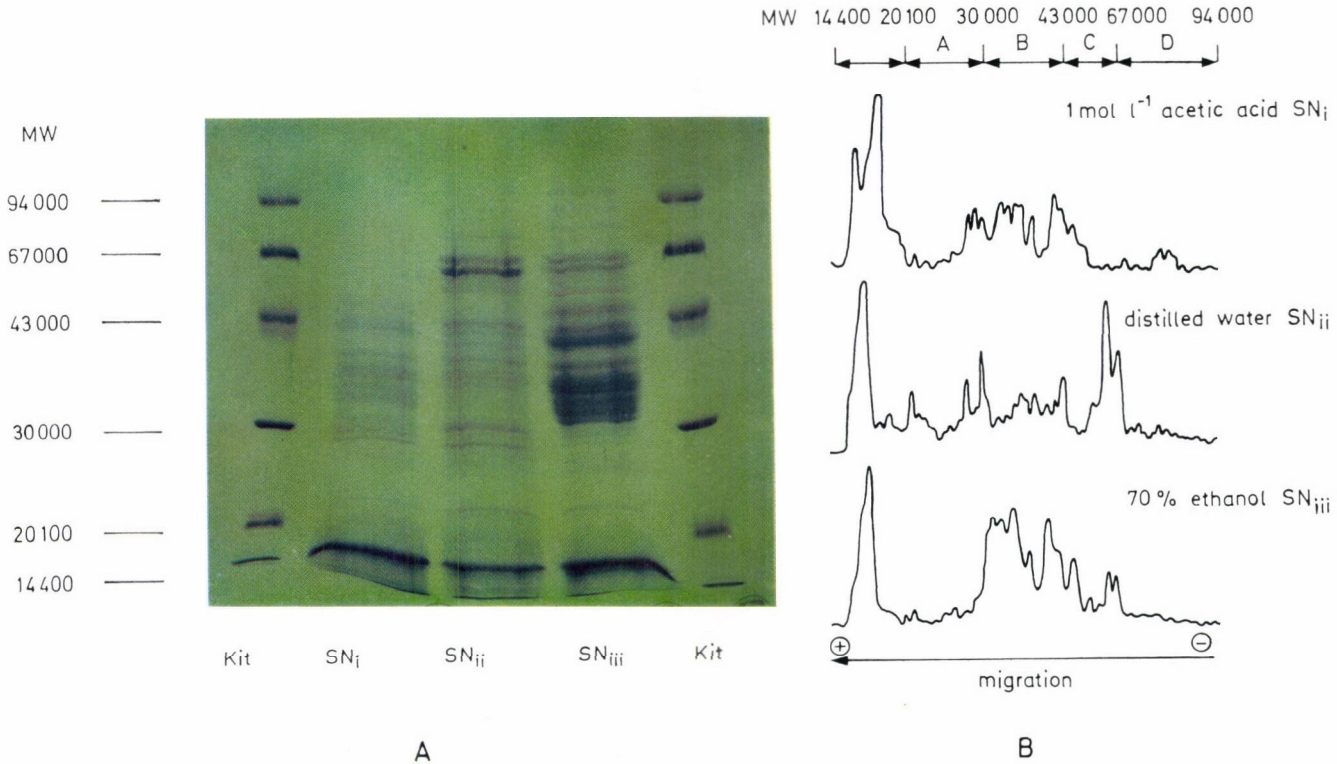


Fig. 2. Protein electrophoresis (A) and densitometer tracings of the electrophoregrams (B) of 1 mol l^{-1} acetic acid (SNi), distilled water (SNii) and 70% ethanol (SNiii) soluble fractions from unfermented bread doughs. Polyacrylamide (PA) slab gel electrophoresis using dissociating (sodium dodecyl sulfate)–discontinuous buffer system (SDS-PAGE) was performed on 10% PA, following the procedure described by LAEMMLI (1970). Blue Brillant Coomassie stained bands were identified by comparing measured light absorption at 630 nm in a LKB densitometer with stained molecular weight standards kit (MW: 14 000, 20 100, 30 000, 43 000, 67 000, 94 000 daltons), product of Pharmacia

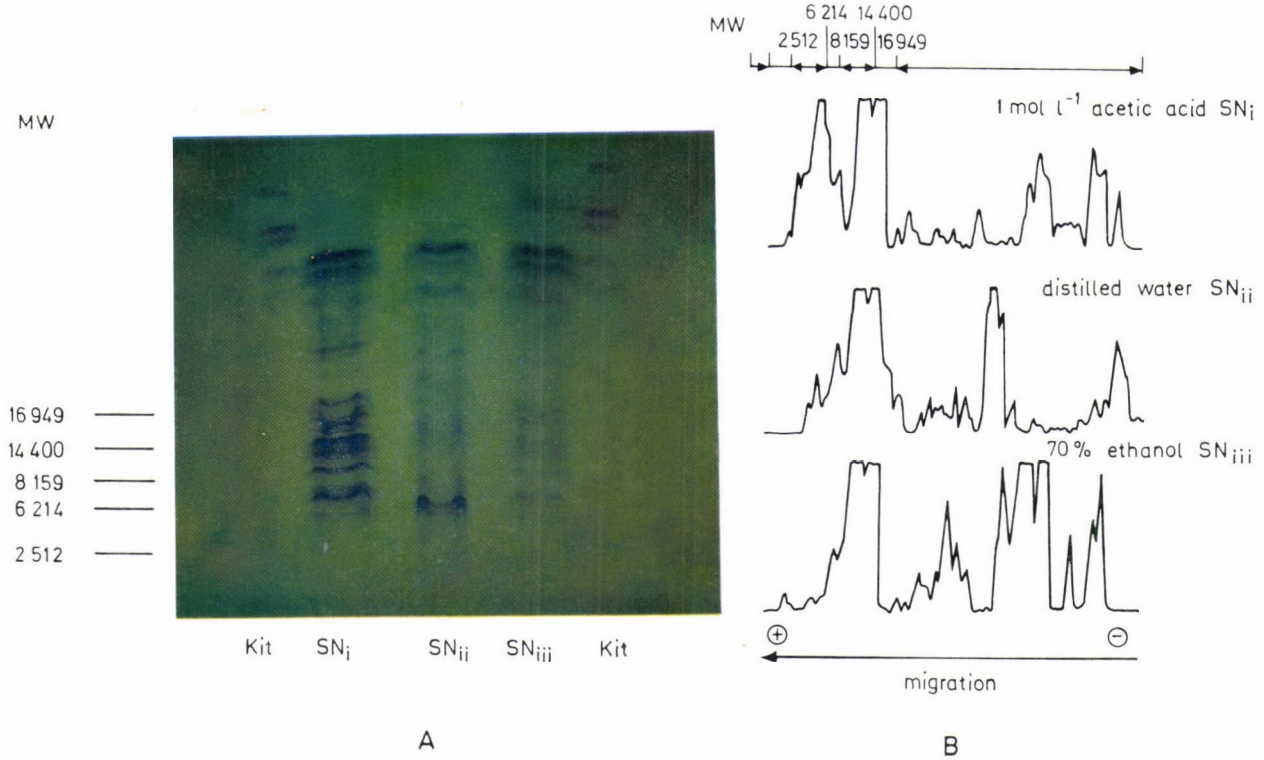
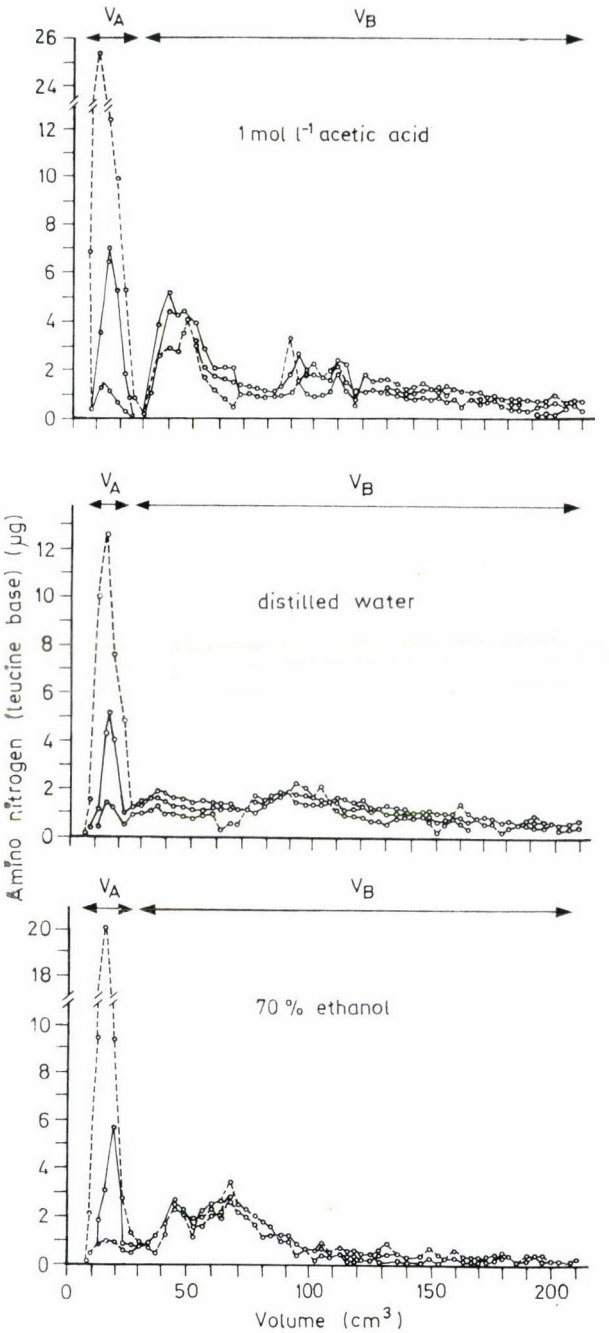


Fig. 3. Polypeptides electrophoresis (A) and densitometer tracings of the electrophoregrams (B) of 1 mol l^{-1} acetic acid (SNi), distilled water (SNii) and 70% ethanol (SNiii) soluble fractions from unfermented bread doughs. Polyacrylamide (PA) linear gradient slab gel electrophoresis was carried out from 10 to 18% PA (gel containing 7 mol l^{-1} urea, acrylamide: bisacrylamide ratio of 20 : 1, using LAEMMLI (1970) discontinuous buffer system), following the prccedure described by HASHIMOTO and co-workers (1983). Blue Brillant Coomassie stained bands were identified by comparing measured light absorption at 630 nm in a LKB densitometer with stained molecular weight standards kit (MW: 2512, 6214, 8159, 14400, 16949 daltons), product of Merck


V_A
V_B
1 mol l⁻¹ acetic acid
distilled water
70 % ethanol
Amino nitrogen (leucine base) (µg)
Volume (cm³)
0
50
100
150
200

separated SN into numerous bands of different electrophoretic mobility (Fig. 2) which reveals quantitative and qualitative differences among proteins depending on the solvent. These are most prominent in the gliadin subfraction (B, C), though they are also important in the albumin and globulin subfractions (A). Polyacrylamide gel electrophoresis of 10–18% notably improved resolution of low molecular weight polypeptide fraction of SN (Fig. 3). Thus, in the zone of MW estimated to be below 16 949 daltons, some electrophoretic bands are resolved, which show prominent qualitative differences among extracts, particularly in the zone of MW $<$ 8 159. Acetic acid extract shows the highest content in LMW peptides (Fig. 4).

2.2. Purification

The elution profiles of the extracts show a clear separation of peptides and amino acids by ligand exchange chromatography (Fig. 4). Peptides are eluted preferentially in the V_A zone, as it is shown by the bigger area under the AN curve with respect to that under the AAN curve, and by the prominent increase in this area after hydrolysis. α-amino acids are eluted mainly in the V_B zone. Eluates from V_A and V_B constitute, respectively, the purified soluble peptide fraction (PSN) and the soluble amino acid fraction (ASN).

2.2.1. Peptides. AN content of PSN shows differences in the extractability of LMW peptides from dough depending on the solvent type. The highest value corresponds to the acetic acid extract (1.14 mg per 100 g dough, d.b.). It is 17.5% lower in the ethanol extract and 26.3% lower in the distilled water extract. In addition, the acetic acid extract is richer in peptides. AAN content of PSN is lower for the acetic acid extract (0.02 mg per 100 g dough, d.b.) than for distilled water (0.32 mg) and ethanol (0.33 mg) extracts.

2.2.2. Amino acids. Amino acids are also extracted most exhaustively by acetic acid. AN content of ASN is higher in this extract (5 mg per 100 g dough, d.b.), in distilled water it is 13.8% lower, and 40.5% lower in the ethanol extract. Differences in AAN content in ASN follow the same trend, although

Fig. 4. Ligand exchange elution diagrams for low molecular weight peptides and amino acids of 1 mol l^{-1} acetic acid, distilled water and 70% ethanol purified soluble fractions from unfermented bread doughs. Aliquots of each fraction collected from the Sephadex 6–10 column were combined freeze-dried and redisolved in cm^3 of 50 mol l^{-1} $Na_2B_4O_7$ buffer, pH 11, and then applied to a column (length: 40 cm, diameter: 15 mm) paked with Cu^{2+}-Sephadex (bed: 1.5 × 11 cm). Samples were eluted following the chromatographic conditions described by ROTHENBÜHLER and co-workers (1983); Cu^{2+} was removed from the eluates by cation exchange chromatography on Chelex-100 resin. V_A: elution volume of low molecular weight peptides, V_B: elution volume of amino acids,(○): primary amino nitrogen content, (●): α-amino nitrogen of free amino acids, (—): before and (---): after acid hydrolysis carried out in evacuated sealed tubes with 0.5 cm^3 6 N HCl at 110 °C for 22 h

those are lower with respect to the ethanol extract. The highest AAN value corresponds to acetic acid extract (4.36 mg per 100 g dough, d.b.); it is 13% and 35% lower in the distilled water and ethanol extracts, respectively.

The isolation in the purest form of LMW peptides from dough and the determination of their content (expressed as AN) in the purified extracts allows an approximate calculation of their content in the extract, in relation to the bread dough. If it is assumed that the average MW of the isolated peptide fraction (MW $<$ 700 daltons) in the acetic acid extracts is 400 daltons, its concentration in the extract is approximately 32 mg per 100 g dough, d.b. Analogously, if an average MW of the amino acids in the acetic acid extract is assumed to be 122 daltons, their estimated content (from AN content) in the extract is 43 mg per 100 g dough, d.b.

3. Conclusions

Results obtained have shown the existence of statistically significant quantitative, and in some case qualitative differences in amino acids, peptides and proteins extracted from bread dough depending on the solvent tested. Analyses performed on SN have shown not only differences in extractability, but in the selectivity of the nitrogen fractions. Thus, ratios of AN and AAN content to those of TS and TN have shown that distilled water and acetic acid are more selective for the extraction of LMW nitrogen components. Electrophoretic analysis of SN has allowed to obtain data concerning electrophoretic behaviour of polypeptides and proteins from bread dough, for each solvent. Results have shown that differences among extracts in TN content are associated with differences in extractability of gliadins. Likewise, they have revealed that acetic acid is much more effective than distilled water or ethanol for the extraction of polypeptide fraction $<$ 8 159 daltons.

Purification of SN has allowed to estimate LMW peptide content of bread dough, for which no data have been published until now. This is important in relation to the amino acid content and seems to indicate that peptides play an important role in bread production. At the same time, it has revealed a variation in peptides and amino acids extractability as a function of the solvent used, which had not been investigated until now for bread dough. AN content from PSN has shown that acetic acid, among the three solvents tested, offers the greatest extraction capacity for LMW peptides. Differences between acetic acid and distilled water are similar to those observed for the extraction of peptides from flour (Jones & Carnegie, 1969). AAN content from PSN is preferentially associated with α-amino butyric acid, β-alanine and, in part, cysteine, which are coeluted in model experiments with peptide fraction (Rothenbühler et al., 1979), although N-glycylpeptides also give ninhydrin positive reaction for the determination of AAN (Doi et al., 1981).

Acetic acid also shows greater extraction capacity for free amino acids (expressed as AN or AAN) from dough. This is followed by distilled water and by ethanol; differences between the latter two show the same trend at those observed in the extraction of amino acids from wheat (WALISZEWSKI et al., 1982), although they are more prominent in dough.

The extraction with 1 mol l^{-1} acetic acid and the purification according to the sequence: ultrafiltration, cation exchange, molecular exclusion and ligand exchange chromatography is considered to be an advisable way for the isolation and purification of low molecular weight peptides from bread dough, whose fractionation and identification is underway now.

Literature

DOI, E., SHIBATA, D. & MATOBA, T. (1981): Modified colorimetric ninhydrin methods for peptidase assay. *Analyt. Biochem., 118*, 173–184.

EL-DASH, A. A. (1971): The precursors of bread flavour: effect of fermentation and proteolytic activity. *Bakers Dig., 45*, (6), 26–31.

EL-DASH, A. A. & JOHNSON, J. A. (1970): Influence of yeast fermentation and baking on the content of free amino acids and primary amino groups and their effects on bread aroma stimuli. *Cereal Chem., 47*, 247–259.

FARIDI, H. A. & JOHNSON, J. A. (1978): Saltine cracker flavor — Part I. Changes in organic acids and soluble nitrogen constituents of cracker sponge and dough. *Cereal Chem., 55*, 7–15.

HASHIMOTO, F., HORIGOME, T., KANBAYASHI, M., YOSHIDA, K. & SUGANO, H. (1983): An improved method for separation of low molecular weight polypeptides by electrophoresis in sodium dodecyl sulfate polyacrylamide gel. *Analyt. Biochem., 129*. 192–199.

HORVAT, R. J., HAWKINS, N. G. & PENCE, J. (1962): Amino acids in fermented bread doughs. *Fd Sci., 27*, 583–586.

JONES, I. K. & CARNEGIE, P. R. (1969): Isolation and characterisation of disulphide peptides from wheat flour. *J. Sci. Fd Agr., 20*, 54–60.

KRETOVICH, V. L. & PONOMAREVA, A. N. (1961): Participation of amino acids in the reaction of melanoidine formation in bread baking. *Biochem., 26*, 237–243.

LAEMMLI, V. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature, 227*, 680–685.

LINKO, Y. Y. & JOHNSON, J. A. (1963): Changes in amino acids and formation of carbonyl compounds during baking. *Agr. Fd Chem., 11*, 150–152.

MORIMOTO, T. (1966): Studies on free amino acids in sponges, doughs and baked soda crackers and bread. *J. Fd Sci., 31*, 736–741.

PONOMAREVA, A. N., KRETOWICH, V. L., KAREVA, I. I. & YAKUBCHIK, T. (1964): Dynamics of free amino acid level in the course of wheat bread preparation. *Biochem., 29*, 283–287.

ROTHENBÜHLER, E., WAIBEL, R. & SOLMS, J. (1979): An improved method for the separation of peptides and α-amino acids on copper-sephadex. *Analyt. Biochem., 97*, 367–375.

SPACKMAN, D. H., STEIN, W. H. & MOORE, S. (1958): Automatic recording apparatus for use in the chromatography of amino acids. *Analyt. Chem., 30*, 1190–1206.

THE UNITED STATES OF AMERICA PHARMACOPEIA (1975): Nitrogen determination. Method No. I. USP, pp. 905–906.

WALISZEWSKI, K., NOWAKOWSKA, K. & SKUPIN, J. (1982): Porownanie roznych method ekstraksji wolnych aminokwasow z materialu roslinnego i zwierzecego. (Comparison of different methods of extracting free amino acids from plant and animal materials.) *Bromatol. Chem. Toksykol., 15*, 327–332.

Acta Alimentaria, Vol. 18 (1), pp. 65–69 (1989)

A NEW SPICE FROM TURKEY: *LASER TRILOBUM* (L.) BORKH.

I. INHIBITORY EFFECT ON FOOD-CONTAMINATING MICROORGANISMS

A. AKGÜL

Department of Food Science and Technology, Faculty of Agriculture, Atatürk University, 25170 Erzurum. Turkey

(Received: 4 February 1988; accepted: 25 April 1988)

The sensitivity of thirty foodborne microorganisms to a new spice, the fruit of *Laser trilobum* (L.) Borkh., the essential oil, and one of its main constituents, perillaldehyde, were studied in culture media. At ground spice concentrations of 1.0–4.0%, bacteria and yeasts were more sensitive than moulds tested. The essential oil at 0.01–0.1% exerted a weak inhibitory effect on all organisms. A concentration of 0.1% of perillaldehyde inhibited completely the growth of the moulds and bacteria studied, except *Pseudomonas aeruginosa*, while it showed slight activity on yeasts. In general, ground spice exhibited a higher antibacterial and anti-yeast activity than essential oil and perillaldehyde. It was also concluded that the antimicrobial property of the spice depended also on non-volatile constituents.

Keywords: antimicrobial effect, *Laser trilobum* fruit, perillaldehyde, spice essential oil

Laser trilobum (L.) Borkh. [syn: *Laserpitium trilobum* L., *Siler trilobum* (L.) Crantz] is a perennial herbaceous plant (fam. Umbelliferae). It is native to S. E. Europe, Caucasia, Iran and Asia Minor, and grows wildly in most regions of Turkey. The fruits are oblong, glabrous, dorsally compressed, with several ridges and 7–8×3–4 mm (DAVIS, 1972). Dried fruits are used as a flavouring agent for some foods, in a similar manner to cumin, and are called "Kefe Kimyonu" in Turkish. The plant is cultivated commonly in the Bartin region (Zonguldak, N. Turkey) (BAYTOP, 1963).

The fruit is used as spice and known in Turkey. No investigations have been reported on its composition and antimicrobial properties, although some physical and chemical constants of the essential oil had been determined in the 1920s (GUENTHER, 1953).

Many studies have been conducted on the prevention of the microbiological spoilage of foods by the addition of natural materials, especially spices, instead of synthetic additives (PRUTHI, 1980; SHELEF, 1983). Our preliminary study indicated that the essential oil of *L. trilobum* fruit contained about 60% limonene and 32% perillaldehyde as its main components.

This paper reports the sensitivity of common foodborne microorganisms to the spice, its essential oil and perillaldehyde, in laboratory media.

1. Materials and methods

1.1. Plant material and essential oil

Fruits were obtained from plants cultivated in the Bartin region. The plants were identified botanically as *L. trilobum* by the Botany Department, Atatürk University, Erzurum, and voucher specimens were deposited. A pale yellow essential oil was obtained at a yield of 8.1% (v/w) by hydrodistillation using a Clevenger-type apparatus (NIGAM, 1966).

1.2. Antimicrobial activity tests

The effects of the ground fruit, essential oil and perillaldehyde (kindly supplied by E. J. Brunke, Dragoco GmbH, Holzminden, FRG) on the growth of thirty common foodborne microorganisms including bacteria, moulds and yeasts (see Tables) were studied. Stock cultures were kindly supplied by the Bundesanstalt für Fleischforschung, Kulmbach, FRG (bacteria), USDA, Northern Regional Research Center, Peoria, USA (moulds) and CSIRO, Food Research Laboratory, North Ryde, Australia (yeasts).

Inocula were prepared by transferring a loopful of the stock cultures into tubes and incubating at 35 °C for 18–24 h (bacteria) and at 30 °C for 48 h (yeasts). Nutrient broth was used as culture medium. Inocula and the spore suspensions of moulds were prepared as described previously (AKGÜL & KIVANÇ, 1988).

Sterile plates were separately inoculated with ca. 10^6 cells cm^{-3} of bacteria, 10^6 spores cm^{-3} of moulds and 10^6 cells cm^{-3} of yeasts. Ground spice was sterilized together with the media; essential oil and perillaldehyde concentrates were dissolved in 0.1 cm^3 quantities of ethanol and then were added to the sterilized media. Sterilized media containing specified amounts of the test materials were poured onto the inoculated plates. Nutrient agar (pH 7.0), potato dextrose agar (pH 5.6) and yeast extract–malt extract–peptone–glucose agar (pH 5.5) were used as growth media for bacteria, moulds and yeasts, respectively. The plates were incubated at 35 °C for 24–48 h (bacteria) at 22 °C for 30 days (moulds) and at 30 °C for 4 days (yeasts). Growth was visually (microscopically for *Geotrichum candidum*) evaluated by comparison with control plates. All experiments were done in duplicate.

2. Results

An important inhibitory activity was not shown by 1.0% ground spice against moulds and yeasts, 0.01–0.05% essential oil against all test organisms, 0.01% perillaldehyde against bacteria and moulds. Only 0.1% perillaldehyde exhibited some effect on yeasts (data not shown).

Ground spice had the highest antibacterial activity. The effect of perillaldehyde was also marked. The antibacterial effect of essential oil was weak *P. aeruginosa* was the most resistant bacterium to all the tested materials (Table 1).

The antifungal effect of ground spice was very small. Perillaldehyde, especially at 0.1% concentration, showed strong inhibitory action on moulds.

Table 1

Inhibitory effects of L. trilobum *fruit, its essential oil and perillaldehyde on the growth of food-contaminating bacteria*

Bacteria	Ground spice (%)				Essential Oil (%)	Perillaldehyde (%)	
	0%	1.0	2.0	4.0	0.1	0.05	0.1
Bacillus cereus	XX	—	—	—	—	X	—
Bacillus subtilis	XX	X	—	—	XX	X	X
Enterobacter aerogenes	XX	X	—	—	XX	X	X
Escherichia coli	XX	XX	X	X	XX	X	—
Proteus vulgaris	XX	—	—	—	X	X	—
Pseudomonas aeruginosa	XX	XX	X	X	XX	XX	XX
Salmonella typhimurium	XX	X	X	—	—	X	—
Staphylococcus aureus	XX	—	—	—	XX	X	—
Streptococcus faecalis	XX	X	—	—	X	X	—
Vibrio parahaemolyticus	XX	X	—	—	XX	X	—

XX: complete growth; X: slight growth; —: no growth

Table 2

Inhibitory effects of L. trilobum *fruit, its essential oil and perillaldehyde on the growth of food-contaminating moulds*

Moulds	Ground spice (%)				Essential Oil (%)		Perillaldehyde (%)			
	0%		2.0		4.0		0.1		0.05		0.1	
	M	S	M	S	M	S	M	S	M	S	M	S
Aspergillus flavus	2	3	2	4	2	7	2	3	15	19	>30	>30
Aspergillus niger	2	3	2	3	2	7	2	5	6	10	>30	>30
Aspergillus oryzae	2	3	2	3	2	8	2	3	16	>30	>30	>30
Aspergillus ustus	2	3	2	5	2	7	2	10	6	10	>30	>30
Fusarium moniliforme	2	3	2	11	2	19	2	7	5	6	>30	>30
Geotrichum candidum	2	4	2	11	2	20	2	5	>30	>30	>30	>30
Mucor sp.	2	3	2	7	2	18	2	7	4	5	>30	>30
Penicillium chrysogenum	2	3	2	6	2	8	2	6	6	7	>30	>30
Penicillium oxalicum	2	3	2	7	2	7	2	5	8	11	>30	>30
Rhizopus sp.	2	3	2	7	2	18	2	7	4	8	>30	>30

M: mycelium growth; S: sporulation
The numbers express the duration of inhibition in days

Table 3

Inhibitory effects of L. trilobum *fruit, its essential oil and perillaldehyde on the growth of food-contaminating yeasts*

Yeasts	Essential				
	Ground spice (%)			Oil (%)	Perill-aldehyde (%)
	0.0%	2.0	4.0	0.1	0.1
Brettanomyces intermedius	XX	X	—	X	X
Candida tropicalis	XX	XX	XX	XX	XX
Candida utilis	XX	XX	X	XX	XX
Debaryomyces hansenii	XX	X	—	XX	X
Kloeckera apiculata	XX	X	—	X	X
Kluyveromyces fragilis	XX	X	—	XX	XX
Pichia membranaefaciens	XX	X	X	XX	X
Rhodotorula rubra	XX	XX	X	XX	XX
Saccharomyces cerevisiae	XX	X	X	XX	XX
Schizosaccharomyces pombe	XX	X	—	X	X

For legends see Table 1

Essential oil was found to have no antifungal activity. Ground spice and essential oil retarded only the sporulation of the moulds tested. *G. candidum* was the most sensitive mould (Table 2).

Ground spice, particularly at 4.0% concentration, exhibited the highest inhibitory effect on yeasts tested. Essential oil and perillaldehyde had little anti-yeast activity. *Brettanomyces intermedius* and *Schizosaccharomyces pombe* were the most sensitive yeasts tested, while the most resistant ones were *Candida* species (Table 3).

3. Conclusions

In an earlier study, similar results were obtained for the inhibitory effect of *L. trilobum* essential oil against some bacteria, using different test methods (KIVANÇ & AKGÜL, 1986).

It was reported that phenolic essential oil components had the strongest inhibitory effects on microorganisms, followed by aldehydes and ketones (KNOBLOCH et al., 1986). This is in agreement with our tests using perillaldehyde as the inhibitory agent. KURITA and co-workers (1981) also reported that perillaldehyde exhibited a clearly inhibitory action on the growth of different moulds.

An investigation of the in vitro anti-yeast activity of various spice essential oils demonstrated that *Kloeckera apiculata* appeared to be the most sensi-

tive and *Kluyveromyces fragilis* the most resistant of the yeasts tested (CONNER & BEUCHAT, 1984). In our study, the sensitivity of both these yeasts were found to be moderate. There may be substantial differences among strains of yeasts with regard to sensitivity to anti-growth effects of essential oils (PRUTHI, 1980; SHELEF, 1983).

It can be concluded from the results that ground spice had the most potent inhibitory effect on the growth of bacteria and yeasts. In contrast, perillaldehyde showed a very strong inhibitory effect on moulds, unlike the other materials tested. Ground spice is used locally are some pickles, meat and soup dishes in Turkey. The amounts used in foods is usually 1.0–3.0%. According to FISCHETTI (1980), perillaldehyde, a cyclic terpene aldehyde, is also a common flavouring additive for many food end-products, usually at 50–500 ppm levels. Concentrations at which this spice and perillaldehyde exert a desirable antimicrobial effect in foods may be higher than customarily used for flavouring purpose; but coupled with other agents and/or treatments, they may aid in the control of microbiological spoilage.

Finally, the results also suggest that the antimicrobial properties of *L. trilobum* fruit probably depends also on its non-volatile components. Further studies are in progress on the related compounds of the fruit and the other components of the essential oil.

Literature

AKGÜL, A. & KIVANÇ, M. (1988): Inhibitory effect of selected Turkish spices and oregano components on some common foodborne fungi. *Int. J. Fd Microbiol., 6*, 263—268.

BAYTOP, T. (1963): *Türkiye'nin tibbi ve zehirli bitkileri* (Medicinal and poisonous plants of Turkey). Istanbul Univ. Ecza. Fac., Istanbul, p. 295.

CONNER, D. E. & BEUCHAT, L. R. (1984): Effects of essential oils from plants on growth of food spoilage yeasts. *J. Fd Sci., 49*, 429–434.

DAVIS, P. H. (1972): *Flora of Turkey and the East Aegean Islands*. Vol. 4. University Press, Edinburgh, p. 513.

FISCHETTI, F., Jr. (1980): Natural and artificial flavours. — in: FURIA, T. E. (1980): *Handbook of food additives*. Vol. 2. CRC Press, Boca Raton, pp. 229–338.

GUENTHER, E. (1953): *The essential oils*. Vol. 4. D. Van Nostrand, New York, p. 752.

KIVANÇ, M. & AKGÜL, A. (1986): Antibacterial activities of essential oils from Turkish spices and citrus. *Flavour Fragr. J. 1*. 175–179.

KNOBLOCH, K., WEIGAND, H., WEIS, N., SCHWARM, H.-M. & VIGENSCHOW, H. (1986): Action of terpenoids on energy metabolism. — in: BRUNKE, E.-J. (1986): *Progress in essential oil research*. Walter de Gruyter, Berlin, pp. 429–445.

KURITA, N., MIYAJI, M., KURANE, R. & TAKAHARA, Y. (1981): Antifungal activity of components of essential oils. *Agric. Biol. Chem., 45*, 945–952.

NIGAM, I. C. (1966): Analysis of volatile oils. — in: CHATTEN, L. G. (1966): *Pharmaceutical chemistry*. Dekker, New York, p. 439.

PRUTHI, J. S. (1980): *Spices and condiments: chemistry, microbiology, technology*. Academic Press, New York, pp. 16–68.

SHELEF, L. A. (1983): Antimicrobial effects of spices. *J. Fd Safety, 6*, 29–44.

Acta Alimentaria, Vol. 18 (1), pp. 71–77 (1989)

DEFT-TECHNIQUE IN THE ANALYSIS OF FOODS

I. PRETREATMENT AND ENZYMATIC DIGESTION

N. E. JAEGGI[a], R. BRUNNER and W. SCHMIDT-LORENZ

Department of Food Science
Swiss Federal Institute of Technology
CH-8092 Zürich, Universitätstrasse 2. Switzerland

(Received: 12 February 1988; accepted: 28 June 1988)

For the analysis of foods by the direct epifluorescent filter technique (DEFT) a variety of homogenised foods was rendered filterable by sedimentation, centrifugation and prefiltration through nylon mesh filters. Centrifugation of food suspensions resulted in good clarification but poor microorganism recovery. A combined sedimentation and prefiltration process yielded good clarification and also good microorganism-recovery. The elimination of food debris was further enhanced by adding enzymes and the surfactant Triton. Protease readily degraded cream, yoghurt, minced meat and frozen fish, whereas the application of amylase was only partially successful in degrading precooked pasta, frozen peas, patisserie and ground pepper.

Keywords: epifluorescent microscopy, food, filterability, prefiltration, enzymatic digestion

The direct epifluorescent filter technique (DEFT) has become a very valuable and widely used method for the fast enumeration of microorganisms in milk and milk products (COUSINS et al., 1979; PETTIPHER et al., 1980; PETTIPHER & RODRIGUEZ 1981; 1982; BECK & HEHIR 1982). The method is easily adjustable for other foodstuffs, provided they can be rendered filterable (WOOD 1982; PETTIPHER & RODRIGUEZ 1982). SHARPE and co-workers (1979) and ENTIS (1982) have carried out extensive work on the filterability of different foods. However, filterability alone does not necessarily guarantee a perfect DEFT-count, since food debris and/or somatic cells can make the counting of microorganisms difficult or impossible. Thus the removal of debris from food suspensions is necessary for microscopic techniques. This has been attempted applying various mechanical pretreatments and enzymatic digestions to different foodstuffs.

1. Materials and methods

1.1. Preparation and pretreatment of food suspensions

1.1.1. Food samples. Minced meat, frozen peas, natural yoghurt and precooked pasta (Spaetzli) were obtained from local stores. Samples of 250 g were

[a] Present address: Kant. Labor, Frohbergstr. 3. CH-9000 St. Gallen

inoculated with equal amounts of *Candida guilliermondi* (LMZ 1800) and *Staphylococcus aureus* (LMZ 174) to a concentration of 10^3–10^4 cfu g^{-1} and stored overnight at room temperature. Thus the final concentration was approximately 10^6–10^7 cfu g^{-1} (Exception: Natural yoghurt was not inoculated). All foods were thoroughly mixed and divided into 10 g samples. (LMZ = Laboratory for food microbiology. Swiss Federal Institute of Technology).

1.1.2. Colony count. Triple dilutions and standard pour plate counts were performed using dilution solution (1.0 g soypeptone Oxoid L 44, 8.5 NaCl, 1000 cm^3 deionized H_2O) and standard method agar (BBL 11638). Colonies were counted after 3 days incubation at 30 °C. Yoghurt was plated onto Elliker agar (Difco 0974 with 12 g Oxoid agar L 13) and incubated anaerobically at 30 °C.

1.1.3. Pretreatments

— *Homogenisation*: 10 g food-sample and 90 cm^3 dilution solution were "stomached" for 30 s.

— *Sedimentation*: After allowing to stand the homogenized food samples for 5, 15 and 30 min the supernatant was taken for standard plate count and further processing.

— *Prefiltration*: After 5 min sedimentation 20 cm^3 supernatant solution was filtered through previously cut and sterilized disks of nylon mesh filters with 5 μm, 27 μm, 105 μm pore size. (5 μm Nytal HD 5 Seidengazefabrik Thal, 27 μm Polyman PES Seidengazefabrik Zürich, 105 μm Estal PE 105 Seidengazefabrik Thal). The filtration apparatus consisted of a Millipore Multisampler Type 1225 on a jet stream vakuum of 39 kPa. Before and after prefiltration the standard plate count was determined.

— *Centrifugation*: 100 cm^3 of homogenized food suspension were centrifuged at 1000 g, 2000 g, 3000 g, respectively, without holding time. Before and after the process the colony counts were performed as described above.

1.1.4. Optical density measurements. A Perkin Elmer 124 double beam spectrophotometer was used to measure the absorption of pretreated food suspensions at 60 nm. The optical density of pea solutions was determined directly, all other foods were 1 : 10 diluted.

1.2. Enzymatic digestion

1.2.1. Food samples. All food samples i. e. pasteurized cream 35% fat content, natural yoghurt, minced meat, frozen fish, precooked pasta (Spaetzli), frozen peas, ground pepper, canned ravioli in tomato sauce, patisserie (nut rolls and chocolate-iced sponge cake) were purchased locally.

Table 1

Enzyme and surfactant treatment of milk, cream and pretreated food homogenates

Food	Prefiltrate (cm^3)	Enzyme	Surfactant	Time (min)	Temp. (°C)
Pasteurized cream	1	0.5 cm^3 Bactotrypsin	10 cm^3 Triton 0.1%	15	50
Natural yoghurt	2	2.0 cm^3 Bactotrypsin	2 cm^3 Triton 0.5%	10	50
Minced meat	2	0.5 cm^3 Protease	2 cm^3 Triton 0.5%	10	50
Frozen fish	2	0.5 cm^3 Protease	2 cm^3 Triton 0.5%	10	50
Precooked pasta (Spaetzli)	2	0.5 cm^3 Amylase	2 cm^3 Triton 0.5%	10	50
Frozen peas	1	0.5 cm^3 Amylase	2 cm^3 Triton 0.5%	10	50
Ground pepper	2	0.5 cm^3 Amylase	2 cm^3 Triton 0.5%	10	50
Canned ravioli	1	0.5 cm^3 Protease 0.5 cm^3 Amylase	2 cm^3 Triton 0.5%	10	50
Patisserie (Nussgipfel)	2	0.5 cm^3 Protease 0.5 cm^3 Amylase	2 cm^3 Triton 0.5%	10	50
Patisserie (Mohrenkopf)	1	0.5 cm^3 Protease 0.5 cm^3 Amylase	2 cm^3 Triton 0.5%	10	50

Enzymes: Bactotrypsin, Difco, 20% in deionized water
Amylase XI A, Sigma, 20% in Tris-HCl buffer pH 7.0
Protease XIV, Sigma 0.1% in Tris-HCl buffer pH 7.6

1.2.2. Pretreatment. All samples were homogenized, the solutions then sedimented for 5 min and prefiltered through 27 μm nylon mesh filters as described above. Exceptions: Pasteurized cream was not pretreated and ground pepper was only filtered through 5 μm nylon mesh as well as pretreated as above.

1.2.3. Enzyme and surfactant treatment. Table 1 shows surfactant, enzyme and time, temperature combinations used to degrade the various foods.

1.2.4. Filter performance and epifluorescent microscopic examination. After enzymatic digestion the food solutions were filtered through 0.6 μm Nucleopore filters, stained with acridine orange and the filters then prepared for microscopic examination, all according to the method of PETTIPHER and co-workers (1980). Observations on the filterability and the appearance of the microscopic field were conducted.

2. Results

2.1. Pretreatment of food suspensions

The microorganism recovery rate and optical density measurements as a means of determining the degree of clarification are shown in Fig. 1. Recovery rates were calculated as a percentage of the plate count after 5 min sedimentation, which was taken to be 100%.

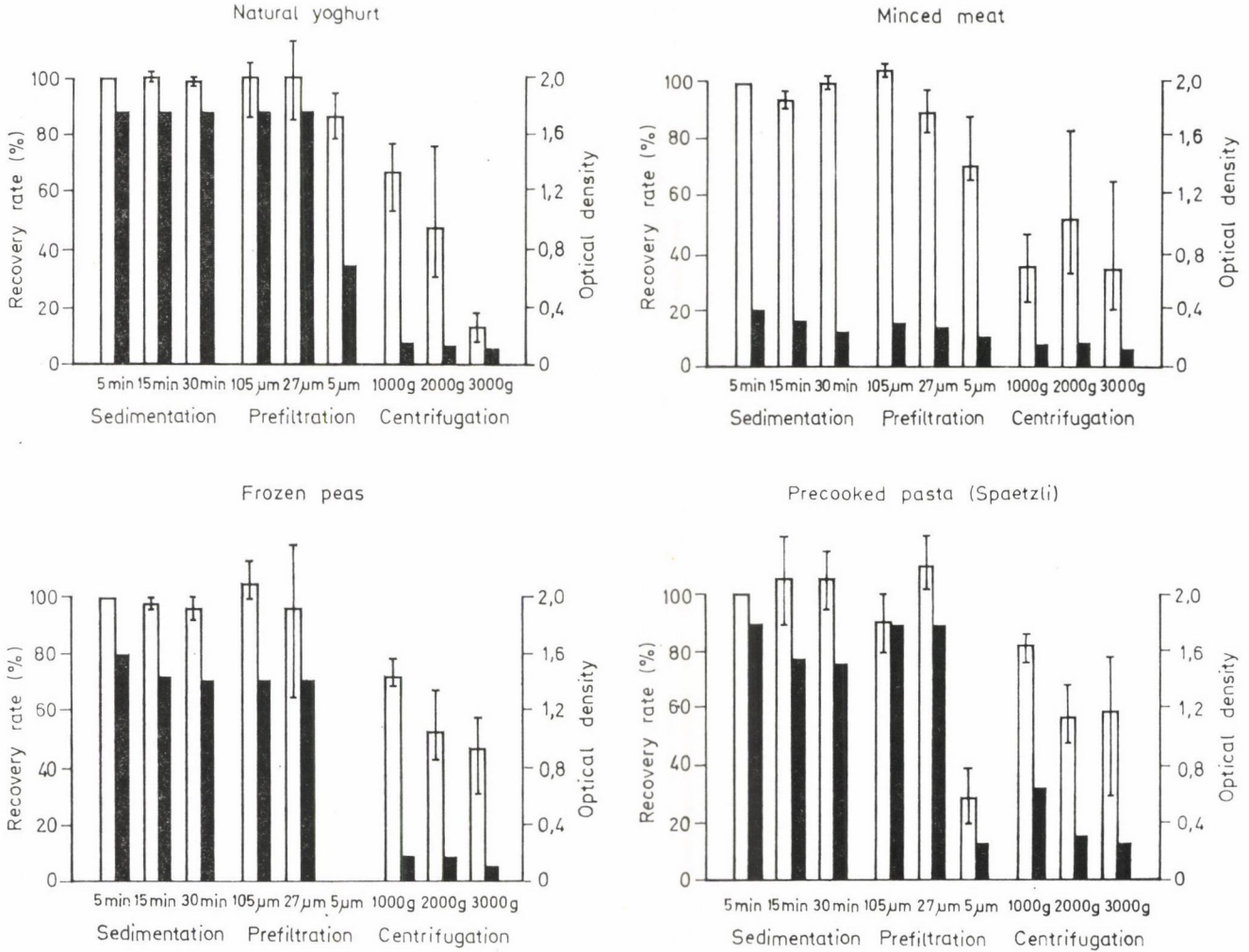


Fig. 1. Optical density (■) and microorganism recovery rate (□) after clarification by sedimentation, prefiltration and centrifugation

Best clarification was obtained by centrifugation, but the loss of microorganisms was between 20 and 70%, exceeding 80% in the case of yoghurt at 3000 g.

Filtration through 5 μm nylon mesh filters produced good clarification but also an unacceptable loss of bacteria. Only small quantities of food solutions could be filtered, which in the case of frozen peas were insufficient for the determination of the plate count. There was little difference in optical density after 27 μm and 100 μm pore size filtration as compared to 15 and 30 min sedimentation. Microorganism recovery rates were also good to excellent. However, optical density measurements did not reveal that the sedimentation process failed to completely eliminate suspended food-particles, which subsequently may settle on the filter and impede microscopic cell counts.

2.2. Enzymatic digestion

The success of enzymatic digestion and surfactant treatment was examined qualitatively under the epifluorescent microscope after filtering and dyeing the samples according to PETTIPHER and co-workers (1980).

— Pasteurized cream: 2 cm^3 could not be rendered filtrable with the described method and had to be reduced to 1 cm^3.

— Natural yoghurt: The Nucleopore filter tended to get clogged, which resulted in a longer contact time of the acridine orange and the other reactants. Therefore, the amount of isopropanol had to be reduced to prevent decolorisation of the stained microorganisms.

— Minced meat, frozen fish: Good clarification was obtained, no meat or fat particles were observed under the epifluorescent microscope.

— Precooked pasta (Spaetzli): Filterability of the 2 cm^3 of food suspension was satisfactory, however, many food particles disturbed the microscopic image.

— Frozen peas: Only 1 cm^3 food suspension was filterable, food debris were observed under the microscope.

— Ground pepper: Pretreated pepper (27 μm nylon mesh) could not be rendered filtrable. After filtration through 5 μm nylon mesh pepper passed through the Nucleopore filter without further treatment, but microorganism recovery was less than 1%.

— Canned ravioli: Few food particles were still present upon microscopic analysis.

— Patisserie: Large, red fluorescent food particles made microorganism detection impossible (nut rolls) or difficult (chocolate-iced sponge cake).

3. Discussion

The determination of the total cell count with the DEFT-technique without pretreatment is practicable only for water and other liquids like wine, beer or some fruit juices (KOCH et al., 1986). All other foodstuffs require a maximum possible volume of suspension to be passed through the smallest possible pore size. Even if filterability is attained food particles may clogg the filter surface or contribute to considerable problems in the staining and counting process. In addition, the loss of microorganisms has to be minimized. In an attempt to meet all these requirements different pretreatment steps were tried:

— Homogenisation: SHARPE and co-workers (1979) recommended the homogenisation in a stomacher for 30 s suggesting that microorganisms were actually washed from the food.

— Centrifugation: STANNARD and WOOD (1983) obtained good results by centrifuging fresh meat at 2000 g for up to 10 s. Perhaps microorganism recovery might be improved with a centrifuge with extremely short acceleration and retardation times.

— Prefiltration: PETERKIN and SHARPE (1981) recommended the use of 111 μm filters, whereas ENTIS (1982) used prefilters with 10 μm and in a few cases even 5 μm pore size. PETTIPHER and RODRIGUEZ (1982) seem to have successfully applied 5 μm pore size prefilters for a variety of different foodstuffs. Present data show a poor microorganism recovery and a poor filter performance. Pore sizes of 27 μm and 105 μm yielded satisfactory clarification as determined by optical density measurements, and the loss of microorganisms was negligeable.

In order to enhance filterability through 0.6 μm pore size Nucleopore filters and to remove food debris still present after pretreatment, enzymatic and surfactant treatment proved to be indispensable. Pasteurized cream, yoghurt, minced meat and frozen fish were readily degraded by applying buffered protease and the surfactant Triton at 50 °C for 10–20 min. For milk products Bactotrypsin was suitable whereas Protease XIV was preferable for meat and fish. According to SHARPE and co-workers (1979), the interaction of protease and surfactants with the food at different temperatures are not fully understood. Triton is believed to be able to disperse non-dissolved fat and thus reduce filterability. Yet BECK and HEHIR (1982) trace the increased filterability of milk back to the lysis of somatic cells by Triton.

The digestion of peas, pasta and pepper using amylase was not entirely satisfactory. Probably starch and pieces of plant cells are not sufficiently degraded by amylase. SHARPE and co-workers (1979) noticed a decrease of the filterability of potatoes when using Triton and PETERKIN and SHARPE (1980) suggested the use of Tween 80 instead. A combination of amylase, cellulase, pectinase and Tween 80 might improve the results.

The use of a combination of proteinase and amylase was successful for canned ravioli and to some extent for patisserie. In order to enhance the degradation of starch granules, they might have to be swelled by a short time heating process.

Literature

BECK, G. & HEHIR, A. F. (1982): The rapid fluorescence method for counting bacteria in milk. *Austr. J. Dairy Techn., 37*, 66–67.

COUSINS, C. M., PETTIPHER, G. L., MCKINNON, C. H. & MANSELL, R. (1979): A rapid method for counting bacteria in raw milk. *Dairy Ind. Int., 44*, 27–32.

ENTIS, P. (1982): Effect of prefiltration and enzyme treatment on membrane filtration of foods. *J. Fd Prot., 45*, 8–11.

KOCH, H. A., BANDLER R. & GIBSON, R. R. (1986): Fluorescence microscopy procedure for quantitation of yeasts in beverages. *Appl. Envir. Microbiol., 52*, 599–601.

PETERKIN, P. I. & SHARPE, A. N. (1980): Membrane filtration of dairy products for microbiological analysis. *Appl. Envir. Microbiol., 39*, 1138–1143.

PETERKIN, P. I. & SHARPE, A. N. (1981): Filtering out food debris before microbial analysis. *Appl. Envir. Microbiol., 42*, 63–65.

PETTIPHER, G. L., MANSELL, R., MCKINNON, C. H. & COUSINS, C. M. (1980): Rapid membrane filtration — epifluorescent microscopy technique for direct enumeration of bacteria in milk. *Appl. Envir. Microbiol., 39*, 423–429.

PETTIPHER, G. L. & RODRIGUEZ, U. M. (1981): Rapid enumeration of bacteria in heat-treated milk and milk products using a membrane filtration-epifluorescent microscopy technique. *J. Appl. Bact., 50*, 157–166.

PETTIPHER, G. L. & RODRIGUEZ, U. M. (1982): Rapid enumeration of microorganisms in food by the direct epifluorescent filter technique. *Appl. Envir. Microbiol., 44*, 809–813.

SHARPE, A. N., PETERKIN, P. I. & DUDAS, I. (1979): Membrane filtration of food suspensions. *Appl. Envir. Microbiol., 37*, 21–35.

STANNARD, C. J. & WOOD, J. M. (1983): The rapid estimation of microbial contamination of raw meat by measurement of ATP. *J. Appl. Bact., 55*, 429–438.

WOOD, R. J. (1982): New developments in the rapid estimation of microbial populations in foods. — in: DAVIES, R. (Ed.) *Developments in food microbiology.* Applied Science Publishers, London.

Acta Alimentaria, Vol. 18 (1), pp. 79–88 (1989)

DEFT-TECHNIQUE IN THE ANALYSIS OF FOODS

II. AGREEMENT WITH THE STANDARD PLATE COUNT

N. E. JAEGGI[a], R. BRUNNER and W. SCHMIDT-LORENZ

Department of Food Science
Swiss Federal Institute of Technology
CH-8092 Zürich, Universitätstrasse 2. Switzerland

(Received: 12 February 1988; accepted: 28 June 1988)

A variety of foods was analysed by the direct epifluorescent filter technique (DEFT) after attaining filterability through prefiltration and enzymatic digestion. DEFT-counts of pasteurized milk, pasteurized cream, minced meat and frozen fish were in agreement with the results of the standard plate count, whereas correlation was low in precooked pasta, frozen peas, patisserie and canned ravioli. Reasons for low correlations between the two methods are discussed. Variation of the DEFT-count is predominantly caused by the uneven distribution of micro-organisms on the membrane filters. A sampling scheme using duplicate filters with 15 counts per filter reduces the standard error of the mean count to below 20%.

Keywords: epifluorescent microscopy, food, total cell count, standard plate count

For enumerating bacteria, the direct epifluorescent microscopy offers the advantages of speed and a relatively high sensitivity over more conventional methods. In water- and environmental-microbiology the technique has been in use for a long time for the determination of total cell counts as opposed to the determination of colony forming units (JONES & SIMON, 1975; HOBBIE et al., 1977; DALEY, 1979). It has since found widespread applications for instance in the simultaneous determination of total cell counts and the respiratory activity of bacteria (ZIMMERMANN et al., 1979) or in the selective enumeration of gram-positive and gram-negative bacteria (RODRIGUEZ & KROLL 1985). In food microbiology particular interest has been shown in substituting the direct epifluorescent filter technique (DEFT) for the standard plate count (PETTIPHER et al. 1980; PETTIPHER & RODRIGUEZ 1981; 1982a; BECK & HEHIR 1982; SUHREN & HEESCHEN 1985; SHAW et al., 1987). For this purpose the DEFT has to be in close agreement or correlation with the standard plate count method. The objective of this study was to assess the suitability of the DEFT-method for a variety of liquid and solid foods after attaining filterability through prefiltration and enzymatic digestion.

[a] Present address: Kant. Labor, Frohbergstr. 3, CH-9000 St. Gallen

1. Materials and methods

1.1. Food samples

All food samples were purchased from local stores. Pasteurised milk, pasteurised cream and patisserie (chocolate-iced sponge cake) were incubated for three days at 22 °C to allow bacterial growth to countable amounts. Frozen fish and frozen peas were thawed and incubated at 22 °C for two days. Canned ravioli (90 g) were stomached together with non-sterile tap water (10 cm^3) for 1 min and incubated for 3 days at 22 °C. Minced meat and precooked pasta (Spaetzli) were processed as they were.

1.2. Colony count

Pasteurised milk and pasteurized cream were thoroughly shaken and triplicate dilutions and pour plate counts were then performed (Standard Methods Agar BBL 11638). All other foods were homogenized in a stomacher for 30 s (10 g food and 90 cm^3 dilution solution, 1 g soypeptone Oxoid L44, 8.5 g NaCl, 1000 cm^3 deionized H_2O). After 5 min sedimentation in sterile 100 cm^3 glass bottles 3×1 cm^3 supernatant were taken for triplicate dilutions and subsequent spread plate counts on Standard Methods Agar (BBL 11638). Colonies were counted after 1, 2 and 3 days at 30 °C. Colony forming units (cfu) per gram or per cm^3 were calculated as the geometric mean of triplicate parallel counts.

1.3. Microscopic DEFT-count

1.3.1. Pretreatment. Homogenized food samples were poured into sterile 100 cm^3 glass bottles for 5 min sedimentation. 20 cm^3 supernatant were filtered through 27 μm nylon mesh filters (Seidengazefabrik Zürich). Milk, cream and prefiltered food-samples were treated with enzymes and Triton as shown in Table 1.

1.3.2. DEFT-count according to Pettipher and co-workers. Eight samples of pasteurized milk were processed according to PETTIPHER and co-workers (1980), i. e. filtration through Nucleopore filters (0.6 μm pore size), staining with acridine-orange 0.025% (w/v) and Tinopal (Ciba-Geigy AG) 0.025% (w/v) in 0.1 mol l^{-1} citrate-NaOH buffer, pH 6.6 for 2 min. Then the filters were rinsed with 2.5 cm^3 citrate-NaOH buffer 0.1 mol l^{-1}, pH 3.0, followed by 2.5 cm^3 of isopropanol. The stained and mounted membranes were examined by means of a Zeiss, Grosses Forschungsmikroskop Universal, filter combination III RS. 10–100 clumps of orange-red fluorescent microorganisms were counted per field of view or per section of an eyepiece graticule.

To obtain the clump count per cm^3 of sample 20 counts per membrane were performed and the arithmetic mean was multiplied by the microscopic and dilution factor.

Table 1

Enzyme and surfactant treatment of milk, cream and pretreated food homogenates

Food	Pre-filtrate (cm^3)	Enzyme	Surfactant	Time (min)	Temperature (°C)
Pasteurized milk	2	0.5 cm^3 Bactotrypsin	2 cm^3 Triton 0.5%	10	50
Pasteurized cream (35% fat content)	1	0.5 cm^3 Bactotrypsin	10 cm^3 Triton 0.1%	15	50
Minced meat	2	0.5 cm^3 Protease	2 cm^3 Triton 0.5%	10	50
Frozen fish	2	0.5 cm^3 Protease	2 cm^3 Triton 0.5%	10	50
Frozen peas	1	0.5 cm^3 Amylase	2 cm^3 Triton 0.5%	10	50
Precooked pasta (Spaetzli)	2	0.5 cm^3 Amylase	2 cm^3 Triton 0.5%	10	50
Canned ravioli	1	0.5 cm^3 Amylase 0.5 cm^3 Protease	2 cm^3 Triton 0.5%	10	50
Patisserie (Mohrenkopf)	1	0.5 cm^3 Amylase 0.5 cm^3 Protease	2 cm^3 Triton 0.5%	10	50

Enzymes: Bactotrypsin, DIFCO, 20% in deionized water
Amylase XI A, Sigma, 20% in Tris-HCl buffer pH 7.0
Protease XIV, Sigma 0.1% in Tris-HCl buffer pH 7.6

1.3.3. DEFT-count modified. Eight samples of pasteurized milk and all other food-homogenates were processed applying the following modifications:

For better contrast, prior to filtration Nucleopore filters were stained with Irgalan-black (Ciba-Geigy AG, 0.2% w/v in 2% acetic acid). The acridine-orange/Tinopal solution was heated to 50 °C before use. The amount of isopropanol was reduced to allow contact times no longer than 15–20 s. For the clump count the number of all green-, orange- and red-fluorescent microorganisms were added together.

1.4. Regression and statistical analysis

For the determination of regression equations four (two) pairs (x, y_{1-4}) were formed with x = log cfu, geometric mean of triple counts per sample, y_{1-4} = log DEFT-count, 4 (2) parallel membranes per sample. In addition, a t-test to calculate the deviation of the slope b from 1 was performed.

The data of eight samples with four membranes per samples of pasteurized milk (method Pettipher), pasteurized milk (method modified), pasteurized cream and minced meat were used to calculate the CV_M [coefficient of variation for a mean number of microorganisms per microscopic field, $CV_M = S/x$] and to compare it to the CV_P [coefficient of variation of a Poisson distribution, $CV_P = 1/\sqrt{x}$] by the Wilcoxon signed-rank test (LINDER, 1979).

The same data were used to performe a two-way analysis of variance (BALAAM, 1972) determining the variance components caused by uneven distribution and counting errors on the membrane compared to sample inhomogeneities and pipetting errors.

2. Results

2.1. Relationship of DEFT-counts and standard plate counts

In Table 2 the regression equations, the coefficients of correlation and the results of the *t*-test are shown. All the foodstuffs yielded good correlation coefficients between 0.91 and 0.99. The slope *b* of the regression lines (Fig. 1) did not deviate significantly from 1 in pasteurized milk, pasteurized cream, minced meat and frozen fish. Because of food particles distorting the microscopic image, it was at times difficult to enumerate microorganisms obtained from frozen pea and patisserie samples. The microscopic image of canned ravioli revealed predominantly red fluorescent yeasts and only small numbers of dimly fluorescent bacteria.

Table 2

Regression equations, coefficients of correlation and t-test ($H_0 : b = 1$) of DEFT-counts versus standard plate counts

Food	Membranes (N)	Regression-equation (y = a + bx)	Coefficient of correlation (r)	*t*-test, $H_0 : b = 1$		
				t_{cal}	$t_{0.05;\,N}$	$t_{0.01;\,N}$
Pasteurized milk (accord. Pettipher)	32	$y = -0.32 + 0.99x$	0.96	0.113	2.037	2.739
Pasteurized milk (method modified)	32	$y = -0.91 + 1.15x$	0.91	1.493	2.037	2.739
Pasteurized cream (35% fat content)	32	$y = -0.41 + 1.15x$	0.97	0.823	2.037	2.739
Minced meat	32	$y = 0.58 + 0.91x$	0.98	2.508	2.037	2.739
Frozen fish	16	$y = 0.63 + 0.90x$	0.98	1.909	2.131	2.947
Frozen peas	14	$y = 1.99 + 0.67x$	0.91	3.727	2.145	2.977
Precooked pasta (Spaetzli)	10	$y = -1.10 + 1.06x$	0.97	0.582	2.228	3.169
Canned ravioli	10	$y = 5.04 + 0.44x$	0.99	—	—	—
Patisserie (Mohrenkopf)	10	$y = 1.22 + 0.81x$	0.98	4.981	2.228	3.169

$t_{cal} = t$ calculated
$t_{0.05\,N}, t_{0.01;\,N}$ = tabulated values at $P \geq 0.05\%$; $P \geq 0.01\%$ probability level, respectively

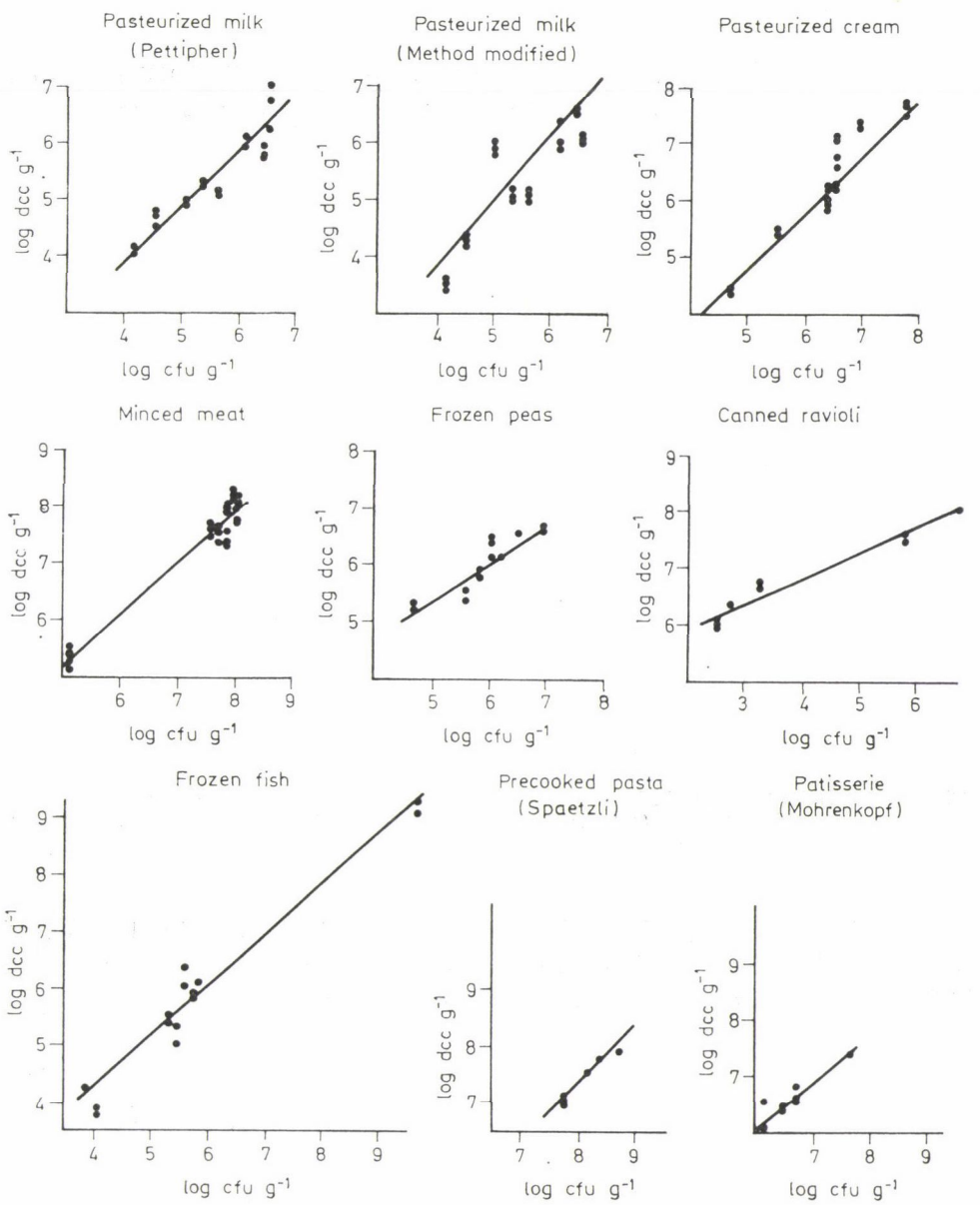


Fig. 1. Correlation between DEFT clump counts (dcc) and standard plate counts (cfu) of various foods

$y = \log$ DEFT clump count g^{-1}

$x = \log$ colony forming units g^{-1}

2.2. Test for Poisson-distribution

The coefficients of variation CV_M of pasteurized milk (DEFT method Pettipher and modified), pasteurized cream and minced meat are situated

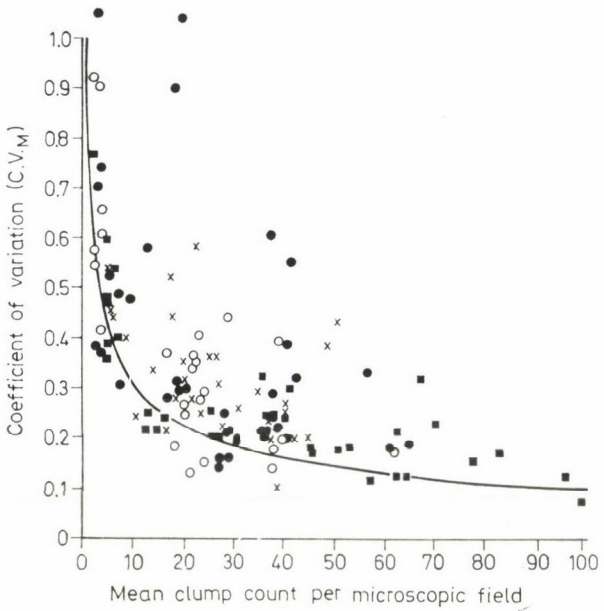


Fig. 2. Coefficients of variation (C.V.$_M$) of minced meat (■), pasteurized cream (×) past. milk, DEFT Pettipher (○), past. milk, DEFT modified (●). Solid line represent expected curve from Poisson distribution

mostly above the CV_P of a Poisson distribution (Fig. 2). Only the CV_M of minced meat appears to follow the CV_P expected from a Poisson distribution. This is also shown by the Wilcoxon signed-rank test in Table 3.

Table 3

Wilcoxon signed-rank test for positive differences of $CV_P - CV_M$

Food	Sum of ranks T for CV_P-CV_M	T theoretical for Poisson distribution
Pasteurized milk (method Pettipher)	58	$P < 0.1$:
Pasteurized milk (method modified)	79.5	$128 < T < 400$;
Pasteurized cream	25	$P < 0.01$:
Minced meat	106	$94 < T < 434$

2.3. Analysis of variance

The analysis of variance reveals (Table 4) that the variance components of total variation between the membrane filters, caused by sample inhomogeneities and pipetting errors is usually between 0%–50%. Hence, variance components on the membranes i. e. distribution inhomogeneities and counting

Table 4

Variation analysis of DEFT-counts of past, milk (method Pettipher) past, milk (method modified), past, cream and minced meat

Food	Variation on membranes, (% of total variance)	Variation between membranes, (% of total variance)	Mean clump count per microscopic field	Standard error 2 membranes; 15 counts (% of mean clump count)
	86	14	8	16
	100	0	41	7
	69	31	37	11
Pasteurized milk	97	3	18	7
(accord. Pettipher)	100	0	27	5
	68	32	21	44
	97	3	61	6
	100	0	4	12
	100	0	3	15
	30	70	30	18
	81	19	20	6
Pasteurized milk	92	8	35	12
(method modified)	78	22	36	13
	95	5	28	7
	95	5	21	5
	85	15	4	16
	20	80	30	33
	66	34	16	18
	87	13	21	15
Pasteurized cream	83	17	44	13
	97	3	6	11
	99	1	28	6
	100	0	40	7
	93	7	23	9
	26	74	68	20
	48	52	35	19
	56	44	81	12
Minced meat	86	14	14	8
	75	25	60	7
	94	6	41	15
	100	0	6	40
	77	23	4	22

errors amount to 50%–100%. Table 4 also shows the calculated standard error of the mean clump for a counting scheme with 2 membranes and 15 counts per membrane. With this counting scheme the standard error drops to 20% or below in 28 of 32 cases.

3. Discussion

The DEFT-counts for pasteurized milk, pasteurized cream, minced meat, frozen fish and precooked pasta were somewhat lower than the standard plate counts. However, the correlation of the two methods was better than described by PETTIPHER and co-workers (1980), PETTIPHER and RODRIGUEZ (1981; 1982a), BECK and HEHIR (1982); GRIFFITHS (1984) and SUHREN and HEESCHEN (1985). This is partly due to the relatively small sample numbers and their homogeneity. The correlation was less accurate in frozen peas, patisserie and canned ravioli. The discrepancies in these foods are probably due to the inadequate clarifying of the food-suspensions. In canned ravioli the DEFT-count was higher because of heat inactivated yeast cells.

A poor relationship and correlation between the two methods can be caused by limitations or inaccuracies in either method. The following methodical errors are most likely to play a predominant role:

— A standard error of 20% is not uncommon in the standard plate count procedure. Further limitations are due to the selectivity of medium and incubation mode (i. e. time, temperature, aerobic, anaerobic).

— Homogenisation. According to CONNOR (1983) the correlation between DEFT- and standard plate counts are improved by additional homogenisation. DEIBEL and BANWART (1982) recorded reductions of bacterial chains to one or two cells by homogenisation. The dilution procedures for the determination of the standard plate count involve mixing by means of a Vortex mixer several times, whereas the DEFT-samples are mixed only once, when enzyme and Triton are added to foodsuspensions. In milk for instance higher bacterial loads increase the influence of different shaking procedures, because chains with up to 25 bacteria are more abundant.

— Prefiltration: Prefiltration of homogenised food samples through nylon mesh filters with 27 μm pore size did not significantly reduce bacterial counts (JAEGGI et al., 1989). Clarification by centrifugation or prefiltration using filters with smaller pore sizes is likely to result in a loss of microorganisms.

— Counting of inactivated cells: The study showed that a differentiation between viable (red fluorescing) and non-viable or inactivated (green fluorescing) cells using acridine orange as a fluorescent dye was not practicable, but also that this was not a prerequisite for using DEFT. QVIST and JACOBSON (1985) reported similar results when testing meat. In canned ravioli, the count-

ing of heat-inactivated red fluorescent yeast cells resulted in a poor correlation of DEFT versus standard plate counts. RODRIGUEZ and KROLL (1986) proposed a modification using Janus Green B. PETTIPHER and RODRIGUEZ (1981; 1982a) detected large amounts of heat inactivated and fluorescent bacteria in evaporated milk and blanched vegetables. Pasteurized cream contained inactivated red fluorescent Micrococci and Streptococci. Cell permeability and dyeing properties of bacteria may be changed by Triton and enzyme treatment according to previous technological treatments. Even products that did not undergo heat treatment may contain inactive cells, for instance deep-frozen foods or various milk products with added starter cultures.

— Distribution of bacteria on membrane: Most of the variance in the presented data is caused by uneven distribution of the bacteria on the filter (50–100%). This compares well with the results of KIRCHMAN and co-workers (1982) and SUHREN and HEESCHEN (1985) who found the variance to be approximately 80%. A standard error below 20% can be obtained by using duplicate filters and counting 15 fields per filter, as is shown by the analysis of variance. For routine controls, when samples are closely related, one membrane and 10 to 15 counts are probably adequate.

It is worthwhile to notice that these sampling schemes are based on a random or Poisson distribution of the bacteria on the membrane (CASSELL, 1965). The results obtained from minced meat were found to be in accordance with the Poisson distribution, but those from pasteurized milk and pasteurized cream were not. KIRCHMAN and co-workers (1982) reported water samples taken from three different locations showing Poisson distribution in only one case. It seems that bacteria in a given substrate are likely to have an influence upon their distribution on the filter. Again, for better results, clumps or chains of bacteria might have to be dispersed by homogenisation or sonification (RODRIGUEZ & KROLL, 1985).

— Operator fatigue: operator fatigue can be a considerable factor in increasing the counting error. The use of a television image analyser, which counts bacteria semi-automatically may further improve the accuracy of the DEFT-method. PETTIPHER and RODRIGUEZ (1982b) reported promising results with raw milk.

Literature

BALAAM, L. N. (1972): *Fundamentals of biometry.* Science and Biology Series 3. George Allen and Unwin Ltd., London.

BECK, G. & HEHIR, A. F. (1982): The rapid fluorescence method for counting bacteria in milk. *Austr. J. Dairy Techn., 37,* 66–67.

CASSELL, E. A. (1965): Rapid graphical method for estimating the precision of direct microscopic counting data. *Appl. Envir. Microbiol., 13,* 293–296.

CONNOR, R. O. (1983): Effect of agitation on bacterial aggregates in pure cultures and raw milk. *J. Fd Prot., 46,* 681–685.

DALEY, R. J. (1979): Direct epifluorescent enumeration of native aquatic bacteria: uses, limitations and comparative accuracy. — in: COSTERTON, J. W. & COLWELL R. R. (Eds.) *Native aquatic bacteria: enumeration, activity and ecology.* American Society for Testing and Materials. (Philadelphia), pp. 29–45.

DEIBEL, K. & BANWART, G. (1982): Comparison of the stomacher with other systems for breaking clumps and chains in the enumeration of bacteria. *J. Fd Prot., 45,* 898–902.

GRIFFITHS, M. W. (1984): Methods for rapid detection of post-pateurisation contamination in cream. *J. Soc. Dairy Techn., 37,* 2–9.

HOBBIE, J. E., DALEY, R. J. & JASPER, R. (1977): Use of Nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Envir. Microbiol., 33,* 1225–1228.

JAEGGI, N. E. BRUNNER, R. & SCHMIDT-LORENZ, W. (1989): DEFT-Technique in the analysis of food: — Part I. Pretreatment and enzymatic digestion. *Acta Alimentaria, 18,* 71–77.

JONES, J. G. & SIMON, B. M. (1975): An investigation of errors in direct counts of aquatic bacteria by epifluorescence microscopy, with reference to a new method for dyeing membrane filters. *J. Appl. Bact., 39,* 317–329.

KIRCHMANN, D., SIGDA, J., KAPUSCINSKI, R. & MITCHELL, R. (1982): Statistical analysis of the direct count method for enumerating bacteria. *Appl. Envir. Microbiol., 44,* 376–382.

LINDER, A. (1979): *Elementare statistische Methoden.* Birkhaeuser Verlag, Basel, pp. 124–125.

PETTIPHER, G. L., MANSELL, R., MCKINNON, C. H. & COUSINS, C. M. (1980): Rapid membrane filtration — epifluorescent microscopy technique for direct enumeration of bacteria in milk. *Appl. Envir. Microbiol., 39,* 423–429.

PETTIPHER, G. L. & RODRIGUEZ, U. M. (1981): Rapid enumeration of bacteria in heat treated milk and milk products using a membrane filtration-epifluorescent microscopy technique. *J. Appl. Bact., 50,* 157–166.

PETTIPHER, G. L. & RODRIGUEZ, U. M. (1982): Rapid enumeration of microorganisms in foods by the direct epifluorescent filter technique. *Appl. Envir. Microbiol., 44,* 809–813.

PETTIPHER, G. L. & RODRIGUEZ, U. M. (1982b): Semi-automated counting of bacteria and somatic cells in milk using epifluorescence microscopy and television image analysis. *J. Appl. Bact., 53,* 323–329.

QVIST, S. H. & JAKOBSON, M. (1985): Application of the direct epifluorescent filter technique as a rapid method in microbiological quality assurance in the meat industry. *Int. J. Fd Microbiol., 2,* 139–144.

RODRIGUEZ, U. M. & KROLL, R. G. (1985): The direct epifluorescent filter technique (DEFT): increased selectivity, sensitivity and rapidity. *J. Appl. Bact., 59,* 493–499.

RODRIGUEZ, U. M. & KROLL, R. G. (1986): Use of the direct epifluorescent filter technique for the enumeration of yeasts. *J. Appl. Bact., 61,* 139–144.

SHAW, B. G., HARDING, C. D., HUDSON, W. H. & FARR, L. (1987): Rapid estimation of microbial numbers on meat and poultry by the direct epifluorescent filter technique *J. Fd Prot., 50,* 652–657.

SUHREN, G. & HEESCHEN, W. (1985): Counting of bacteria with the direct epifluorescent filter technique (DEFT). *Milchwissenschaft, 40,* 283–287.

ZIMMERMANN, R., ITURRIAGA, R. & BECKER-BIRCK, J. (1978): Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. *Appl. Envir. Microbiol., 36,* 926–935.

Acta Alimentaria, Vol. 18 (1), pp. 89–96 (1989)

PROTEOLYTIC EVENTS IN LEGUME STORAGE PROTEINS

M. DURANTI and D. GIANI

Department of Agrifood Molecular Sciences,
University of Milan, 20133 Milan, Via Celoria 2. Italy.

(Received: 24 February 1988; accepted 13 June 1988)

The most significant proteolytic events which modify the storage proteins of lupin (*Lupinus albus*, L.) seed during development to early stages of germination were investigated. Special emphasis was given to globulin 8, the major legumin-like globulin of the seed.

The first event considered is the post-translational breakdown of legumin precursor polypeptides (M_r around 70 000) occurring during seed development which gives rise to two polypeptides usually referred to as legumin acidic (40 000 to 50 000 M_r) and basic (19 000 M_r) subunits.

The second step is the partial proteolysis of the acidic subunits which can be also achieved in vitro by incubation with two endopeptidases from lupin resting seed. The role of this further cleavage is unknown, but it can be related to an altered association-dissociation behaviour of legumin oligomer.

At early stages of germination the breakdown was more extensive and again only the acidic subunits appeared to be affected by the enzymatic proteolysis.

Keywords: proteolysis, storage proteins, *Lupinus albus*, L.

A number of proteolytic events are involved in shaping the storage proteins as they are stored in the mature seeds and in making them available to complete breakdown at germination.

The proteolytic steps which a legume storage protein undergoes from development to maturation and eventually germination of the seed are outlined in Fig. 1.

The first proteolytic event consists in the co-translational removal of the highly hydrophobic signal peptide which according to BLOBEL and DOBBERSTEIN's (1975) hypothesis is required for the segregation of the nascent polypeptide into the lumen of the endoplasmic reticulum (ER). The enzyme involved is an unknown endopeptidase probably located within the ER membrane.

A series of covalent modifications including further limited proteolytic degradation of virtually all the storage proteins (GATEHOUSE et al., 1984), but also formation of disulfide bridges for the legumins (GATEHOUSE et al., 1984) and glycosylation for the vicilins and some legumins (GATEHOUSE et al., 1981; DURANTI et al., 1987b) take place post-translationally before the deposition of the polypeptide as a mature protein in the protein bodies. In the case of

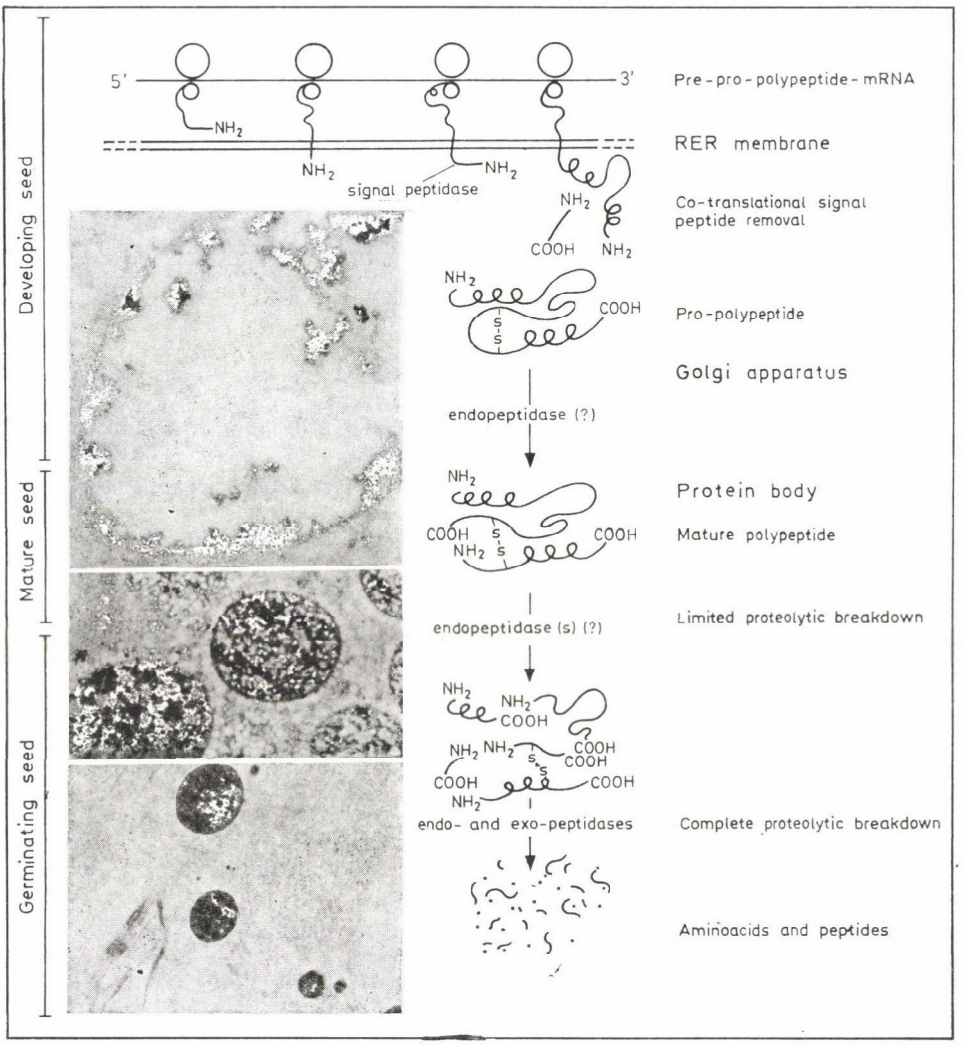


Fig. 1. Proteolytic degradation of storage proteins in legume seeds
Co- and post-translational proteolytic processing of a storage protein in lupin seeds is schematically shown. On the left electromicrographs of lupin protein bodies at different developmental stages

a legumin molecule, like the one schematically drawn in the figure, the pro-polypeptide is proteolytically cleaved in the proximity of a highly hydrophylic region (GATEHOUSE et al., 1984; NIELSEN, 1984). This cleavage gives rise to the acidic and basic legumin subunits which are linked together by disulfide bridge(s). The enzyme(s) involved in this step has not been identified so far.

Further limited proteolytic breakdown of the vicilin and the legumin of a number of legume seeds has been proved to occur (GATEHOUSE et al., 1984).

The role of these latter changes is still obscure but they are thought to be related to a better packaging of the protein in the protein body and/or to a more effective and possibly selective degradation during germination (RYAN & SIMMONS 1981). Free amino acids and small peptides are produced at this stage for the nitrogen and carbon skeleton requirements of the growing seedling. In a recent review by SHUTOV and VAINTRAUB (1987) also the extensive cleavage of the storage protein during germination has been described as a finely regulated mechanism where differential mobilization of the storage proteins may play an important physiological role.

This paper deals with the proteolytic modifications of a selected storage globulin of lupin seed from its synthesis to germination. In vitro proteolytic processing of the same storage protein with endogenous endopeptidases currently under investigation in our laboratory, has also been reported.

1. Materials and methods

1.1. Reagents

All chemicals were the purest available from Merck, Sigma and BioRad.

1.2. Samples

Resting seeds of *Lupinus albus* were of the sweet Multolupa variety with an alkaloid content of 0.05%.

The seeds were germinated and grown under field conditions in the summer months 1987 at the Faculty of Agriculture, University of Milan. Developing seeds were harvested at selected days after flowering (DAF). Seed coats were removed and the dehushed seeds were frozen in liquid nitrogen and stored at −30 °C prior to use.

1.3. Extraction

Single seeds of a given DAF were weighed and then ground in a mortar previously refrigerated in an ice bath in the presence of 60 mmol l^{-1} TRIS-HCl buffer, pH 7.0 containing 4.5% SDS (1 : 3; w : v). Total proteins were extracted at room temperature for 2–3 h under stirring. Insoluble material was removed by centrifugation at 3000 g at room temperature. Fifteen % sucrose and 2% 2-mercaptoethanol were added to the supernatant which contained about 0.5–1 mg of protein per cm^3. The mixture was then heated at 100 °C for 5 min. Aliquots of the extracts at various DAF were analyzed by SDS-PAGE.

SDS-PAGE was carried out in 15% polyacrylamide gels essentially as described by LAEMMLI (1970). The proteins were stained with Coomassie Brillant Blue R250.

Globulin 8, the major legumin-like storage protein of lupin seed, was extracted from mature seeds as described by DURANTI and co-workers (1987b) and from germinating seeds as described by DURANTI and co-workers (1984).

Endopeptidases 1 and 2 were purified from the albumin extract of resting lupin seeds, respectively, as described by DURANTI and CERLETTI (1988) and by GIANI and CERLETTI (1987).

Proteolytic degradation of globulin 8 from mature seeds was carried out with both endopeptidases as described by DURANTI and CERLETTI (1988).

Other methods were according to DURANTI and co-workers (1987b).

2. Results

The SDS-PAGE pattern of the total proteins extracted from developing lupin seeds is shown in Fig. 2. A progressive proteolytic cleavage of the high M_r polypeptides and an increase of the legumin basic subunit of M_r around 20 000 were clearly visible. Vicilin precursor, i. e. the major band of M_r 68 000, underwent more extensive degradation than the legumin precursor. The most

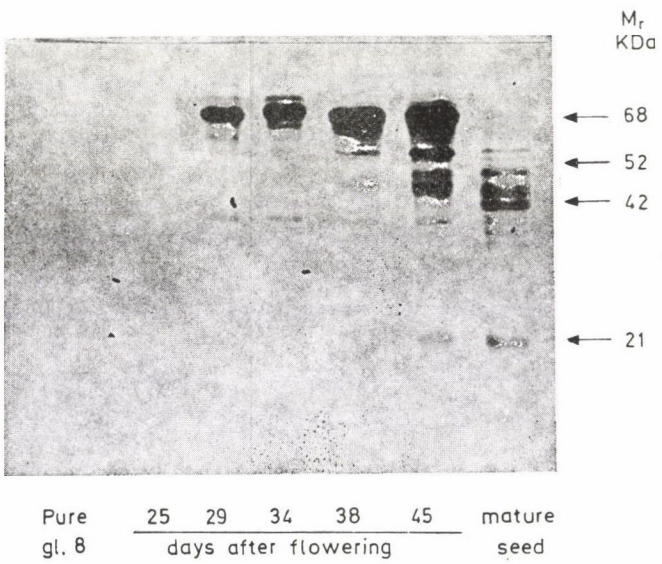


Fig. 2. Changes in total proteins during lupin seed development
Total proteins from lupin seeds extracted at different days after flowering were submitted to SDS-PAGE under reducing conditions. From 5 μg (25 DAF) to 40 μg (45 DAF) of protein were loaded per gel track. Pure globulin 8: 5 μg. Total protein from mature seed: 30 μg

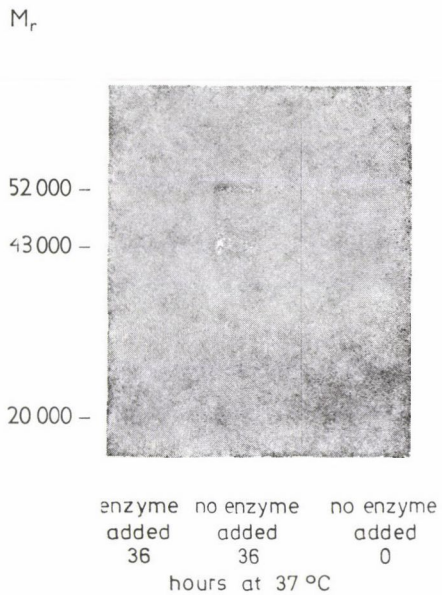


Fig. 3. SDS-PAGE analysis of globulin 8 incubated with endopeptidase 2
Proteolytic cleavage of globulin 8 by endopeptidase 2 is shown in the figure. Globulin 8 : 0.5 mg cm^{-3} in 50 mmol l^{-1} phosphate buffer (pH 8.0), containing 0.15 mol l^{-1} NaCl. Enzyme: 35 μg cm^{-3} in the same buffer as above. Seven μg protein were loaded per gel track

abundant polypeptide fragments were in the range between 50 000 and 40 000. Proteolytic degradation of the precursor polypeptides did not reach completion and some of them were still present in the total proteins extracted from mature dry seed. When Western blotting analysis and subsequent immunodetection with antibodies anti lupin-legumin were used (not shown), the cleavage of the 70 000 M_r legumin pro-polypeptide to give the acidic and the basic subunits of the mature protein was better evidenced.

Part of lupin legumin acidic subunits were further partially degraded in vivo in multiple sites of the polypeptide (DURANTI et al., 1987b, JOHNSON et al., 1985). A similar degradation pattern was obtained by in vitro incubation of the legumin with two endopeptidases, referred to as endopeptidases 1 and 2, isolated from mature lupin seeds (DURANTI & CERLETTI, 1988; GIANI & CERLETTI, 1987). The pattern of degradation of globulin 8 by lupin endopeptidase 2 is shown in Fig. 3. The 20 000 M_r basic subunit was unaffected by the enzyme. The decrease in total intensity of the bands after incubation with endopeptides which were lost during electrophoresis. A less extensive degradation of the acidic subunits of pea and lupin legumin was obtained with endopeptidase 1 (DURANTI & CERLETTI, 1988; DURANTI et al., 1987a).

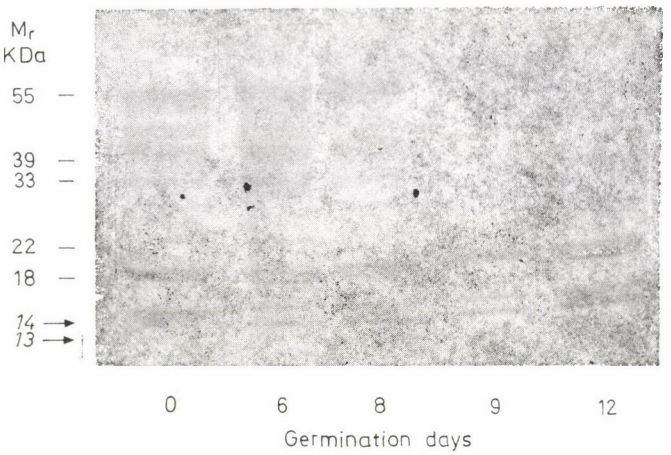


Fig. 4. SDS-PAGE analysis of globulin 8 on different days of germination
Globulin 8 on different days of germination is analyzed by SDS-PAGE under reducing conditions. Ten μg per gel track were loaded

When globulin 8 was extracted from germinating lupin seeds and analyzed by SDS-PAGE a somewhat similar pattern of degradation as that observed in vitro with the mentioned endopeptidases was found (Fig. 4.). Again, only the acidic subunits underwent degradation, whereas the basic ones were unchanged also at advanced stages of germination.

3. Conclusions

Partial proteolytic degradation of lupin storage protein precursors during seed development has been already described in *L. angustifolius* by JOHNSON and co-workers (1985).

Our results confirm this previous finding and set a similar cleavage pattern for another lupin species. Also the major storage protein precursors of pea (GATEHOUSE et al., 1984), broad bean (SHOLTZ et al., 1983) and many other seeds undergo limited fragmentation during development. The observed modifications consist only in the limited post-translational degradation of the globulins.

Co-translational removal of the signal peptide is not detectable by direct measurements, but it has been proved to occur only through cDNA sequence analysis or by in vitro translation using specific mRNA for the storage proteins (see GATEHOUSE et al., 1984 for pea seeds).

The decrease in size of the high M_r subunits of lupin legumin due to partial proteolytic breakdown has been recently related to changes in the aggregation properties of the legumin oligomer. Indeed proteolytically modified

legumin polypeptides were shown to be no more capable of reassociation to the 12 S species as it occurs to the unchanged molecules (DURANTI et al., 1987b).

Proteolysis during germination has been recently recognized as a finely modulated mechanism involving a battery of proteases which display different time of synthesis, various activation processes and different cell location (SHUTOV & VAINTRAUB, 1987). The composition, i. e. aminoacid sequence, presence of covalently linked carbohydrate and degree of amidation of the acidic amino acids, the structure and the level of aggregation of the storage proteins, probably also play an important role in the pattern of degradation during germination.

In all cases the subunits mostly affected by proteolytic attack are the acidic ones, which because of their hydrophylic character (GUERRIERI et al., 1987) and the presence in the legumin of covalently linked carbohydrate (DURANTI et al., 1987b), are probably exposed to the solvent. This is in agreement with the proposed structural models for this molecule (PLIETZ et al., 1987).

In spite of the number of plant proteolytic enzymes discovered (see for a review DALLING, 1986), the physiological events which lead a storage protein to its mature and functional form are still largely undefined.

Therefore further studies are necessary for the comprehension of the mechanisms involved and for an action aimed at controlling or even modifying these processes.

*

The present work has been granted by the Italian National Research Council, Project I. P. R. A. sub-project No. 3. We wish to thank Dr. N. HARRIS for electron micrographs of lupin cotyledons.

Literature

BLOBEL, G. & DOBBERSTEIN, B. (1975): Transfer of protein across membrane. — Part I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane bound ribosomes of murine myeloma. *J. Cell. Biol., 67*, 835–851.

DALLING, M. J. (1986): *Plant proteolytic enzymes.* CRC Press, Boca Raton, Florida. Vol. 1.

DURANTI, M. & CERLETTI, P. (1989): Purification and characterization of a proteinase from the lupin mature seed. *Phytochemistry*, (In press.)

DURANTI, M. CUCCHETTI, E. & CERLETTI, P. (1984): Changes in composition and subunits in the storage proteins of germinating lupin seed. *J. Agric. Fd Chem., 32*, 490–493.

DURANTI, M., GATEHOUSE, J. A., BOULTER, D. & CERLETTI, P. (1987a): In vitro proteolytic processing of pea and jack bean storage proteins by an endopeptidase from lupin seeds. *Phytochemistry, 28*, 627–631.

DURANTI, M., GUERRIERI, N., TAKAHASHI, T. & CERLETTI, P. (1987b): The legumin like-storage protein of *Lupinus albus* seed. *Phytochemistry, 27*, 15–23.

GATEHOUSE, J. A., CROY, R. R. D. & BOULTER, D. (1984): The synthesis and structure of pea storage proteins. *CRC Crit. Rev. Plant Sci., 1*, 287–314.

GATEHOUSE, J. A., CROY, R. R. D., MORTON, H., TYLER, M. & BOULTER, D. (1981): Characterisation and subunit structures of the vicilin storage proteins of pea (*Pisum sativum* L.). *Eur. J. Biochem., 118*, 627–633.

GIANI, D. & CERLETTI, P. (1987): A metalloendopeptidase from quiescent lupin seed. *Abstr. Comm., 33 Italian Biochemistry Society*, Brescia, Italy, p. E53.

GUERRIERI, N., DURANTI, M. & CERLETTI, P. (1987): Associazione oligomerica e transizioni conformazionali nella globuline legumino-simili del seme di lupino. *Abstr. Comm. 33 Italian Biochemistry Society*, Brescia, Italy, p. E54.

JOHNSON, E. D., KNIGHT, J. & GAYLER, K. R. (1985): Biosynthesis and processing of legumin-like storage proteins in *Lupinus angustifolius* (lupin). *Biochem. J., 232*, 673–679.

LAEMMLI, U. K. (1970): Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature (Lond.), 227*, 680–685.

NIELSEN, N. C. (1984): The chemistry of legume storage proteins. *Phil. Trans. R. Soc. 304*, 287–296.

PLIETZ, P., DRESCHER, B. & DAMASCHUN, G. (1987): Relationship between the amino sequence and the domain structure of the subunits of the 11S seed globulins. *Int. J. Biol. Macromol., 9*, 161–165.

RYAN, C. A. & WALKER-SIMMONS, M. (1981): Plant proteinases. — in: STUMPF, P. K. & CONN, E. E.: (Eds.) *Biochemistry of plants*. Academic Press, New York, Vol. 6. pp. 321–350.

SHOLTZ, G., MANTEUFFEL, R., MUNTZ, K. & RUDOLPH, A. (1983): Low-molecular-weight polypeptides of vicilin from *Vicia faba L.* are products of proteolytic breakdown. *Eur. J. Biochem., 132*, 103–107.

SHUTOV, A. D. & VAINTRAUB, I. A. (1987): Degradation of storage proteins in germinating seeds. *Phytochemistry, 26*, 1557–1566.

Acta Alimentaria, Vol. 18 (1), pp. 97—106 (1989)

STUDY ON TASTE SUBSTANCES OF TOMATO

I. ANALYTICAL INVESTIGATIONS INTO THE SUGAR, ORGANIC ACID AND MINERAL CONTENTS

M. PETRÓ-TURZA, I. SZÁRFÖLDI-SZALMA, K. FÜZESI-KARDOS and F. HAJDU

Central Food Research Institute, 1022 Budapest, Herman Ottó út 15. Hungary

(Received: 20 April 1988; accepted: 14 June 1988)

The authors carried out comparative analytical investigations into the sugar, organic acid and mineral contents of two tomato cultivars (one traditional cultivar named "K-549" and another one suitable for machine harvesting and named "Mokka") and the purées manufactured of them. It was established that more than half of the soluble solids content (56.5% for the cultivar "K-549" and 64.1% for the cultivar "Mokka") was made up of glucose and fructose occurring in a ratio near to 1 : 1. A slight decrease in the reducing sugar content was observed during purée manufacturing. Within the organic acids which constituted about 10% of the soluble solids content in fresh tomato samples, citric acid was prevailing in both cultivars while malic acid was present in traces only. Acid content increased upon evaporation due to the formation of pyrrolidone carboxylic acid. Concentrations and qualitative compositions of soluble mineral substances were practically identical in the two tomato cultivars and did not change either during evaporation. Total mineral substances amounted to about 7% of soluble solids content and were composed of about 70% of potassium, about 2% of magnesium, 7.5% of phosphate and 20.5% of chloride. It was established as well that mineral substance content and composition were determined by location and growth conditions but not by cultivar. Out of the parameters studied noteworthy differences between the two cultivars were found in total and soluble solids content as well as in the ratio sugar/acid.

Keywords: tomato, taste substances, sugars, organic acids, minerals

In the present series of papers an account is given of the experimental work aimed at a better knowledge of the taste substances of tomato. For the sake of comparison investigations were carried out in parallel with a so-called traditional cultivar ("K-549") and a new cultivar ("Mokka") suitable also for machine harvesting. Beside the detailed analytical and sensory study of fresh tomatoes, changes occurring in the taste substances during purée manufacturing were followed, too. Prior to starting experimental work, the literature dealing with tomato taste substances had been collected and critically analysed. This work was published in a former paper of ours (PETRÓ-TURZA, 1986–87).

1. Materials and methods

1.1. Materials

The samples to be analysed, i. e., the fresh, ripe tomatoes of the two cultivars of varietal purity and the purées manufactured of them under identical conditions were supplied by the Research Institute of Vegetable Growing (Kecskemét, Hungary).

1.2. Methods

Total solids contents of the homogenized samples were determined by vacuum drying, soluble solids contents were assayed in a similar way from the serum separated by centrifugation.

1.2.1. Methods applied for investigating the sugar content of the samples. Total reducing sugar content of the sera obtained from homogenized samples was determined by the Lane-Eynon method (AOAC, 1960), glucose content by the procedure of KISZEL and SÁNDOR (1962) while fructose content was calculated from the difference of the two measurements. Qualitative detection of the sugar components occurring in the samples as well as their quantitative determination were carried out, beside the classical titrimetric method, also by HPLC. The latter was performed in a Spectra-Physics SP 8700 type apparatus. The column length was 250 mm, the inner column diameter 4.6 mm, the stationary phase LiChrosorb 10 NH_2, the eluent was water, containing 85% acetonitrile with a flow rate of 1 $cm^3\ min^{-1}$.

Detection of the components was carried out with a detector based on the measurement of differences in refractive indices, temperated to 33 °C (range of refractive indices: 1.31–1.44). Separation was performed at room temperature, with D-xylose as internal standard.

1.2.2. Methods applied for studying the organic acid content of the samples. The pH values of the sera obtained from the homogenized samples were determined using a digital pH-meter (Radelkis, type OP-211/1).

1.2.2.1. Measurement of titratable acid content — Several communications are concerned with the determination of the titratable acid content of tomatoes. Measurements are, in general, carried out with a 0.1 mol l^{-1} NaOH solution, however, until reaching different pH values. STEVENS (1972) as well as KOPELIOVITCH and co-workers (1982) titrated to pH 7, KADER and co-workers (1978) as well as SAKIYAMA and STEVENS (1976) to pH 8.1 while BUESCHER and co-workers (1979) continued titration until pH 8.2. In order to determine the effective point of equivalence, we established the titration curve of tomato serum. We intended to determine the end point by a numerical method, calculating the maximum of the differential curve ($\Delta^2E/\Delta V^2 = 0$) (BÁNYAI, 1974). However, we did not succeed in establishing this in an unambiguous way

as 0 values were obtained at several joints for the quotient $\Delta I^2E/\Delta V^2$. Therefore, further on, excess NaOH solution (0.1 mol l^{-1}) was added to the serum and after allowing the mixture to stand for 5 h (this lapse being necessary, according to our experience, for obtaining identical values for parallel measurements), excess lye was titrated back with hydrochloric acid to the inflexion point (pH = 7.5) established from the titration curve. From the slow attainment of the equilibrium state we came to the conclusion that the organic acids of tomatoes can be determined by direct titration but with a great error.

1.2.2.2. Qualitative analysis of individual acid components by Overpressured Layer Chromatography (OPLC) — Among the relatively few communications of the application techniques of this new chromatographic procedure developed in Hungary we did not find any, dealing with the separation of organic acids. Therefore we carried out experiments to select the appropriate layer material, solvent mixture and other parameters (flow rate of the mobile phase, time of run, etc.)

A relatively good separation could be achieved on a Kieselgel G layer activated for 30 min at 105 °C, in a solvent mixture of chloroform–ethyl alcohol–water (45 : 9 : 1) with a solvent flow rate of 0.8 cm^3 min^{-1} and a duration of the run of 12 min. The analyses were carried out in the apparatus type Chrompres 10 manufactured by Labor MIM. Separation was carried out at room temperature. Bromophenol blue indicator prepared according to STAHL's (1967) recipe for bromocresol green, was used for staining.

1.2.2.3. Analysis of individual acid components by HPLC — The sera of the samples as filtered through a Millipore 0.45 μm filter and diluted with distilled water in an appropriate ratio, were directly injected to the HPLC column. Separation was carried out according to BIACS and co-workers (1984). A Liquochrom 2010 type apparatus manufactured by Labor-MIM was used, the column was factory-filled with Chromsil C_{18} (10 μm) stationary phase, was 250 mm long and of 4.6 mm inner diameter. The eluent was a water–methanol (97.5 : 2.5) mixture containing 0.01 mol l^{-1} KH_2PO_4 + 0.75 mmol l^{-1} tetrabutylammonium hydroxide. The pH of the eluent was adjusted with phosphoric acid to 2.75 in the first and to 2.80 in the second series of experiments. The eluent flow rate was 1 cm^3 min^{-1} and the pressure 39 bar throughout the column. The components were detected with an UV detector at 225 nm. Quantitative measurements of citric acid and pyrrolidone carboxylic acid were carried out using calibration curves.

1.2.3. Methods used to determine the mineral substance content of the samples. As the aim of our study was the measurement of the compounds affecting the taste, only those mineral substances were determined which occurred in the serum in a dissolved state. Total mineral content was determined after combustion by gravimetry. Out of the cations, Na, K, Ca and Mg were determined quantitatively in a Pye Unicam type SP 2900 double-beam atomic absorption

spectrophotometer, at the wavelengths corresponding to the elements investigated. Prior to analysis, the samples were subjected to moist digestion performed in a teflon bomb. Subsequently, the determination of K and Na were performed in emission, those of Ca and Mg in absorption.

Phosphorus content of the samples was determined spectrophotometrically, using the method of BUTENKO and KIRSCH (1940) while chloride content was assayed with a chloride ionselective electrode (Radelkis, type OP-Cl-071 1P).

2. Results and discussion

The data relative to total solids content as well as to its soluble and insoluble parts as found in the samples analysed are summarized in Table 1.

Table 1

Solids contents of the two tomato cultivars and of the corresponding purées

Sample	Total solids (g per 100 g)	Soluble solids (g per 100 g)	Insoluble solids[a] (g per 100 g)
"Mokka" fruit	11.25±0.05	7.49±0.03	3.76±0.06
"Mokka" purée	28.26±0.12	23.87±0.07	4.39±0.13
"K-549" fruit	8.24±0.01	5.70±0.04	2.54±0.04
"K-549" purée	23.60±0.11	20.37±0.09	3.23±0.12

The data are mean values and standard deviations of three parallels

[a] Calculated values

The results were according to expectations. Total solids content as well as, within this, the insoluble part were present at a higher percentage in the new tomato cultivar "Mokka" suitable also for machine harvesting than in the traditional cultivar "K-549".

2.1. Qualitative and quantitative composition of the sugar content of the samples

HPLC analysis of sugar components of the samples revealed that their bulk was composed of glucose and fructose. Beside these all the samples contained also sucrose in trace amounts. The results of titrimetric and HPLC investigations are summarized in Table 2.

Total reducing sugar as determined by the Lane-Eynon method (AOAC, 1960) and the joint amount of glucose and fructose as determined by HPLC

Table 2

Sugar contents of the two tomato cultivars and of the corresponding purées

Sample	HPLC method (g per 100 g soluble solids)				Titrimetric method (g per 100 g soluble solids)			
	glucose	fructose	glucose + fructose	ratio of glucose and fructose	glucose	fructose	total reducing sugars	ratio of glucose and fructose
"Mokka" fruit	30±1	35±2	65±2	0.86	35±2	30±2	64.7±0.2	1.2
"Mokka" purée	26±2	34±1	60±2	0.76	34±2	28±2	61.9±0.1	1.2
"K-549" fruit	28±1	29±1	57±2	0.97	33±2	24±2	56.7±0.1	1.4
"K-549" purée	26±1	28±1	54±2	0.93	34±2	21±2	55.1±0.1	1.6

The data are mean values and standard deviations of six parallels

were in good agreement for all the samples. The titrimetric values obtained for glucose were, however, systematically higher and the calculated fructose values accordingly lower than those obtained by HPLC. Just for this reason the glucose–fructose quotients calculated from the data obtained by the two methods, showed lesser or greater variations, too. From the data obtained by the two methods, HPLC results were considered to be reliable with respect to individual components. Accordingly, glucose–fructose ratios in the samples approximated the ratio 1 : 1 but in all the samples fructose values were somewhat higher.

Comparing the values obtained for the two tomato cultivars, it was established that sugar content in the cultivar "Mokka" which contained a higher absolute value of soluble solids was higher, too, than in the traditional cultivar "K-549".

Upon evaporation a slight decrease in sugar content occurred with both tomato cultivars; however, taking into account the errors of measurement, this proved to be significant only for the cultivar "Mokka". The separate examination of the decrease in fructose and glucose contents showed glucose to be decomposed to a somewhat higher extent. On the whole, however, the qualitative and quantitative changes in sugar concentration were not important in either cultivar.

2.2. Organic acid content and composition of the samples

Titratable acid contents expressed as citric acid as well as pH values of the samples analysed are shown in Table 3.

From the two cultivars, acids made up a somewhat greater part of the soluble solids content of the traditional cultivar "K-549" than of the cultivar

Table 3

Titratable acidity of the two tomato cultivars and of the corresponding purées

Sample	Titratable acidity[a]		pH
	(g per 100 g sample)	(g per 100 g soluble solids)	
"Mokka" fruit	0.738±0.002	9.7±0.2	4.31
"Mokka" purée	3.12±0.03	13.1 ±0.1	4.34
"K-549" fruit	0.625±0.003	10.96±0.05	4.31
"K-549" purée	2.76 ±0.01	13.55±0.05	4.41

The data are mean values and standard deviations of three parallels

[a] Calculated as citric acid

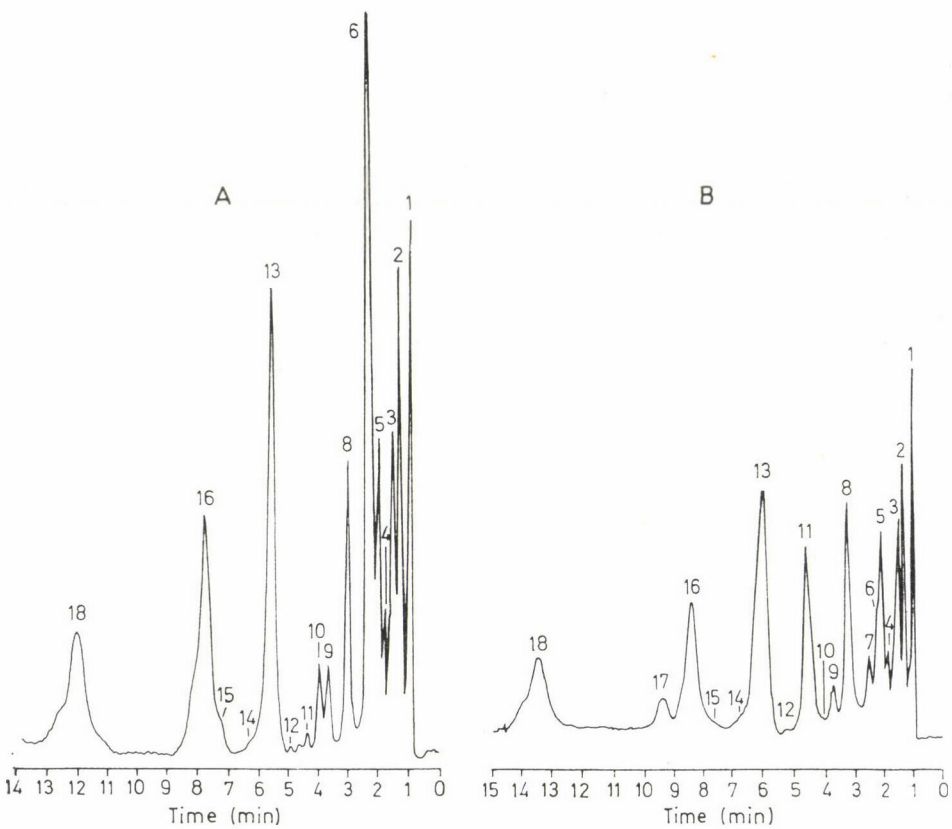


Fig. 1. HPLC separation of organic acids occurring in the tomato cultivar "Mokka" (A) and in the corresponding purée (B), (eluent pH = 2.75). 3. glutamic acid + aspartic acid, 4. galacturonic acid, 5. quinic acid, 6. ascorbic acid, 7. acetic acid, 8. malic acid, 9. tartaric acid, 11. pyrrolidone carboxylic acid, 12. propionic acid, 13. citric acid, 14. fumaric acid, 15. malonic acid

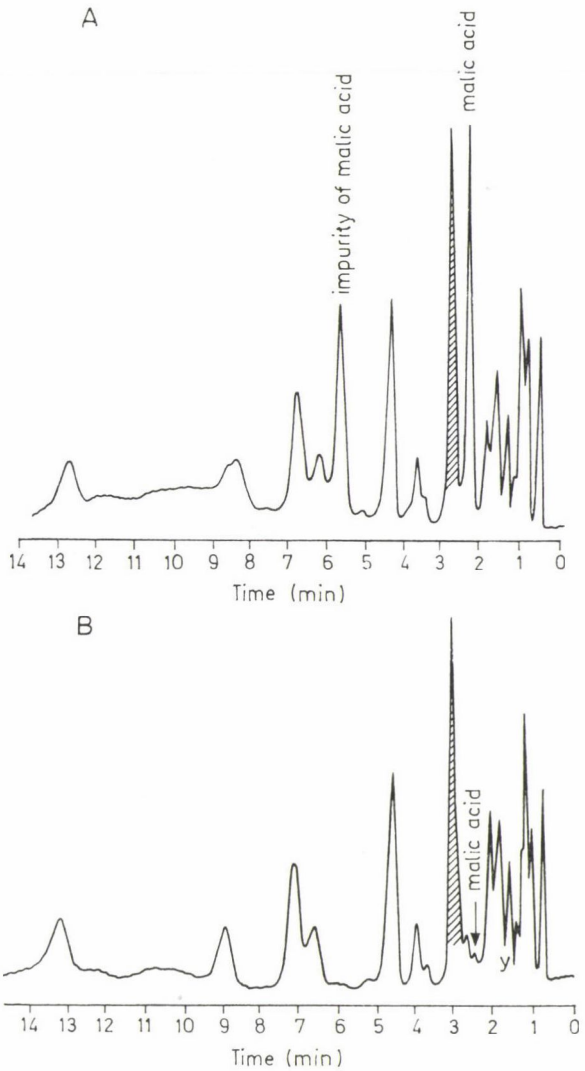


Fig. 2. HPLC separation of organic acids occurring in tomato purée made of cultivar "K-549", with (A) and without (B) malic acid enrichment (eluent pH = 2.80). (The shaded peak eluted together with malic acid when the pH of the eluent was 2.75)

"Mokka". At the same time the pH values of their sera were practically identical. This can be explained by the buffering effect of the sera.

During evaporation acid content increased considerably in both cultivars.

Under the given circumstances by OPLC 3 acid spots were separated from all the samples whereby citric acid was the prevailing component. In all the samples malic acid occurred only in traces while the same applied to

pyrrolidone carboxylic acid in the sera of fresh tomatoes. On the other hand, the spot of this latter acid showed a considerable increase in the purées.

Upon separation of the acid components of the samples by HPLC substantially more peaks appeared in the chromatograms, owing to the higher sensitivity of method. Comparing the chromatograms obtained for the two tomato cultivars, no qualitative differences were found between them. Subsequently we tried to identify the substances present in the peaks, partly by the capacity factors of model acids chromatographed under identical conditions as the samples, and partly by applying the enrichment technique. Figure 1 shows the separation of the organic acids as present in the cultivar "Mokka" and in the purée prepared from it, along with the names of the compounds identified.

The substances of the peaks No. 1, 2, 10, 16, 17 and 18 could not be identified due to the lack of model substances in the required number and satisfactory purity. Subsequently, orientating quantitative measurements were carried out to estimate the citric, malic and pyrrolidone carboxylic acid contents of the samples. The comparison based solely on peak areas was not feasible, due to the specific sensitivity of the detector (e. g. sensitivity was higher, by one order of magnitude, for ascorbic acid than for citric acid). In the case of malic acid results were contradictory to those obtained in OPLC investigations. In order to decide whether the peak in question really corresponded to malic acid, the analysis was repeated with the pH value of the eluent elevated by 0.05 (see para. 1.2.2.3.). Figure 2 shows the separation of the acid components of the purée obtained from the cultivar "K-549" with the eluent of increased pH, with and without enrichment with malic acid.

These chromatograms unambiguously revealed that in the buffer of pH 2.75 as formerly applied, an unknown component was eluted together with malic acid. After having changed the pH of the eluent, this component could be well separated from malic acid. As it can be seen, this latter acid occurred only in minimum amounts in the sample. Similar results were obtained when analysing the other three samples in a similar way. Therefore, from the

Table 4

Citric acid and pyrrolidone carboxylic acid content of the samples

Sample	Citric acid		Pyrrolidone carboxylic acid		Total	
	(g per 100 g fresh weight)	(g per 100 g soluble solids)	(g per 100 g fresh weight)	(g per 100 g soluble solids)	(g per 100 g fresh weight)	(g per 100 g soluble solids)
"Mokka" fruit	0.8 ±0.1	11 ±2	—	—	0.8	11
"Mokka" purée	2.4 ±0.3	10 ±1	0.77±0.07	3.2±0.3	3.2	13.2
"K-549" fruit	0.67±0.05	11.8±0.9	—	—	0.67	11.8
"K-549" purée	2.02±0.08	9.9±0.4	0.61±0.02	3.0±0.1	2.63	12.9

The data are mean values and standard deviations of three parallels

Table 5

Soluble mineral and ash contents of the samples

Sample	Potassium	Magnesium	Phosphate	Chloride	Total ions	Ash
	(g per 100 g soluble solids)					
"Mokka" fruit	5.2±0.6	0.120±0.005	0.530±0.003	1.32±0.06	7.2	7.2±0.2
"Mokka" purée	4.9±0.1	0.150±0.005	0.589±0.003	1.41±0.05	7.1	8.9±0.2
"K-549" fruit	5.2±0.3	0.115±0.006	0.604±0.003	1.23±0.08	7.2	7.0±0.1
"K-549" purée	5.2±0.1	0.128±0.004	0.576±0.006	1.2±0.1	7.1	9.0±0.2

The data are mean values and standard deviations of three parallels

identified individual acids only citric and pyrrolidone carboxylic acids present in higher concentrations were assayed quantitatively. The results are summarized in Table 4.

The results of the analyses revealed that upon evaporation the citric acid content remained practically unchanged or showed a slight decrease. On the other hand, a considerable increase in the amount of pyrrolidone carboxylic acid — present in fresh samples only in traces — could be noticed. As to the rest of the peaks occurring in the chromatograms, the peak corresponding to ascorbic acid decreased to a minimum in concentrates, the area of the unknown peak indicated by number 10 decreased, too, while the areas of the rest of the peaks as related to citric acid, did not show any substantial variation. From all this we arrived to the conclusion that the increase in titratable acid in the concentrates was basically caused by the formation of pyrrolidone carboxylic acid.

2.3. Mineral content and composition of the samples

The results pertaining to the mineral substance content and composition of the samples investigated are summarized in Table 5. In the serum samples calcium and sodium did not occur in measurable amounts.

From the mineral substances of the tomato cultivars only phosphorous or phosphate content showed significant differences. Somewhat more phosphate accumulated in the traditional cultivar "K-549" than in the new cultivar "Mokka". However, to decide if this is really characteristic of the cultivar, samples of several years ought to be studied. Besides, it is not probable that the slight difference observed should cause perceptible differences in either taste or buffer capacity.

The very similar mineral content of the two cultivars might obviously be derived from the fact that their place of origin was the same. At any rate, the results of the investigations indicate that mineral content and composition of tomatoes are essentially not depending on the cultivar.

The mineral contents of fresh tomatoes and of the corresponding purées did not show substantial differences, in agreement with expectations. The significant differences observed in magnesium and phosphorus contents might be explained by the fact that the concentrates were obtained from pulps instead of from sera. In the case of fresh samples the sum of the ions measured was in good agreement with ash content. This proves that measurements embraced all minerals present in higher amounts. The ash content which, in concentrates, was higher than the sum of individual components indicates the solubilization of foreign ions during evaporation.

Considering the percentage distributions of the compositional characteristics of the two tomato cultivars, it can be established, that practically they differed only in the ratios of sugar : acid. This ratio was 100 : 20.9 for the cultivar "K-549", while it was 100 : 17.5 for the cultivar "Mokka".

*

The authors wish to thank Dr. M. Böröcz-Szabó for the measurements of atomic absorption spectrometry, Dr. J. Ujszászi for the HPLC analyses of the sugars and Ms E. Kosztelnik for her active participation in experimental work.

Literature

A.O.A.C. (1960): *Official methods of analysis.* Association of Official Agricultural Chemists, Washington, pp. 426–427.

Bányai, É. (1974): Potenciometria. — in: Erdey, L. & Mázor, L. (Eds.) *Analitikai kézikönyv.* (Analytical handbook.) Műszaki Könyvkiadó, Budapest, p. 405.

Biacs, P., Bodnár, J., Hoschke, Á., Ress, P. & Hajdu, F. (1984): *Oxidation of unsaturated fatty acids in foods.* Paper presented at 4th Annual American–Eastern European Symposium on Liquid Chromatography, Szeged, Hungary.

Buescher, R. W., Hardy, C. & Tigchelaar, E. C. (1979): Characteristics of juice, puree and whole tomatoes processed from nor hybrid fruits. *J. Fd Sci., 44,* 190–192.

Butenko, G. A. & Kirsch, N. V. (1940): Fotokolometricheskoe appredelenie fosfornoi kisloty v udobreniyah. *Zav. Lab., 9,* 555.

Kader, A. A., Morris, L. L., Stevens, M. A. & Albright-Holton, M. (1978): Composition and flavor quality of fresh market tomatoes as influenced by some post-harvest handling procedures. *J. Am. Soc. hort. Sci., 103,* 6–13.

Kiszel, M. & Sándor, A. (1962): Glükóz és fruktóz meghatározása cukorbetegek számára gyártott gyümölcskészítményekben. (Determination of glucose and fructose in diabetic fruit products). *Konzerv Paprikaipar,* (3), 87–89.

Kopeliovitch, E., Mizrahi, Y., Rabinowitch, H. D. & Kedar, N. (1982): Effect of the fruit-ripening mutant genes rin and nor on the flavor of tomato fruit. *J. Am. Soc. hort. Sci., 107,* 361–364.

Petro-Turza, M. (1986–87): Flavor of tomato and tomato products. *Fd Revs Int., 2,* (3), 309–351.

Sakiyama, R. & Stevens, M. A., (1976): Organic acid accumulation in attached and detached tomato fruits. *J. Am. Soc. hort. Sci., 101,* 394–396.

Stahl, E. (1967): *Dünnschicht-Chromatographie.* Springer-Verlag, Berlin, p. 819.

Stevens, M. A. (1972): Relationships between components contributing to quality variation among tomato lines. *J. Am. Soc. hort. Sci., 97,* 70–73.

Acta Alimentaria, Vol. 18 (1), pp. 107–117 (1989)

STUDY ON TASTE SUBSTANCES OF TOMATO

II. ANALYTICAL INVESTIGATION INTO THE FREE AMINO ACID, PEPTIDE AND PROTEIN CONTENTS

M. PETRÓ-TURZA, I. SZÁRFÖLDI-SZALMA, K. FÜZESI-KARDOS and Á. BARÁTH

Central Food Research Institute, 1022 Budapest, Herman Ottó út 15. Hungary

(Received: 20 April 1988; accepted: 14 June 1988)

The authors studied the distribution of N-content in two tomato cultivars ("K-549" and "Mokka") as well as in the purées prepared from them. It was established that in fresh tomatoes more than 70% of the N-content can be derived from the compounds present in the serum in dissolved form. The total amounts of free amino acids and, within these, the percentage ratios of the individual components showed characteristic differences in the two cultivars, however, in both cultivars, glutamic acid and glutamine occurred in the highest quantities. The presence of peptides in the samples was detected, too. Among the amino acid components of these, aspartic and glutamic acids were prevailing. The results obtained with the amino acid analyser for the fractions separated from the samples were in good agreement with nitrogen contents determined with the Kjel-Foss apparatus.

It was established that about 17% of the soluble solids content of tomatoes were made up by free amino acids and 8–11% peptides, while soluble proteins were found in minute amounts only. In our opinion, the considerable deviation of our results from data of the literature can be accounted for by the fact that, for tomatoes, the multiplication factor of 6.25 as generally used, cannot be applied for calculating protein concentration from N-content.

The amount of free amino acids decreased in purées by about 20% as related to fresh fruits while the concentration of peptides increased as a result of partial hydrolysis of insoluble proteins.

Keywords: tomato, taste substances, free amino acids, distribution of N-content

In an earlier review article we gave a survey on our knowledge accumulated so far on the flavor substances of tomatoes (PETRÓ-TURZA, 1986–87). Part of our own experimental work, aimed at the comparative study of the sugar, acid and mineral contents and compositions of two tomato cultivars, was published in Part I of this series of papers (PETRÓ-TURZA et al., 1989). Our present communication reports on the results of the study of qualitative and quantitative analyses of the free amino acids present in the same two cultivars. Moreover, the results obtained for the ratios of small peptides, and dissolved and insoluble proteins in the samples will be dealt with, too.

Akadémiai Kiadó, Budapest

1. Materials and methods

1.1. Materials

The investigations were carried out with two tomato cultivars, the traditional "K-549" and the one named "Mokka" which lends itself also for machine harvesting as well as with the purées manufactured of them under identical conditions. The samples were supplied by the Research Institute of Vegetable Growing (Kecskemét, Hungary).

1.2. Methods

Total nitrogen content of the samples to be analysed was determined in the Kjel-Foss Automatic 16200 type apparatus for crude protein determination. Qualitative and quantitative distribution of the free amino acids present in the samples was studied with the automatic amino acid analyzer Biotronic LC 2000. In order to eliminate, in the mildest way possible, high molecular compounds interfering with amino acid analysis, the sera obtained from the samples were filtered several times through a Gelman 0.3 μm pore size membrane filter. The analysis was carried out according to the standard program described in the manual of the apparatus for the separation of 18 components, using a Na-citrate buffer system. In the given conditions serine, glutamine and asparagine gave a common peak. In order to determine these quantitatively one by one, the separation was carried out also according to another program described in the users' manual of the apparatus for the study of physiological liquids. In this case a Li-citrate buffer was applied.

2. Results and conclusions

2.1. Nitrogen material balance of the samples analysed

A nitrogen material balance was made for ivestigating the distribution of the nitrogen content within the fruit and to determine its changes occurring upon processing. Results are shown in Figs. 1, 2, 3 and 4.

Converting total nitrogen contents of the fresh samples of the tomato cultivars to protein contents, using the normally applied factor of 6.25, yielded somewhat higher results than those reported in the literature (DAVIES & HOBSON, 1981) and found earlier by us (PETRÓ-TURZA & KEVEI-PICHLER, 1975). This can be explained by an increase in the nitrogen supply of the soil as well as with the higher solids content of the samples investigated in the present study.

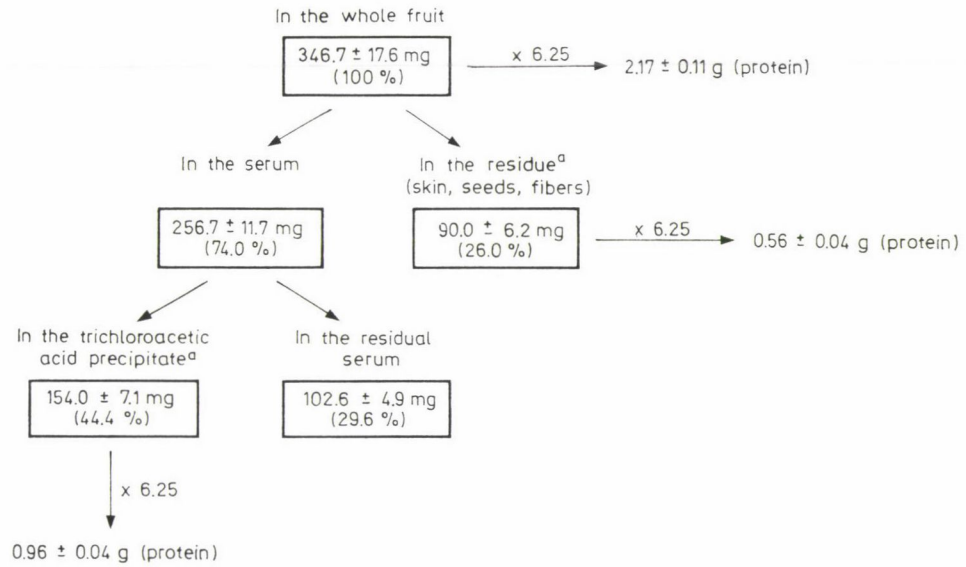


Fig. 1. Distribution of nitrogen content in 100 g fresh fruit of the tomato cultivar "Mokka" as determined by Kjel-Foss analyzer. The data are mean values and standard deviations of three independent experiments.
[a] Calculated values

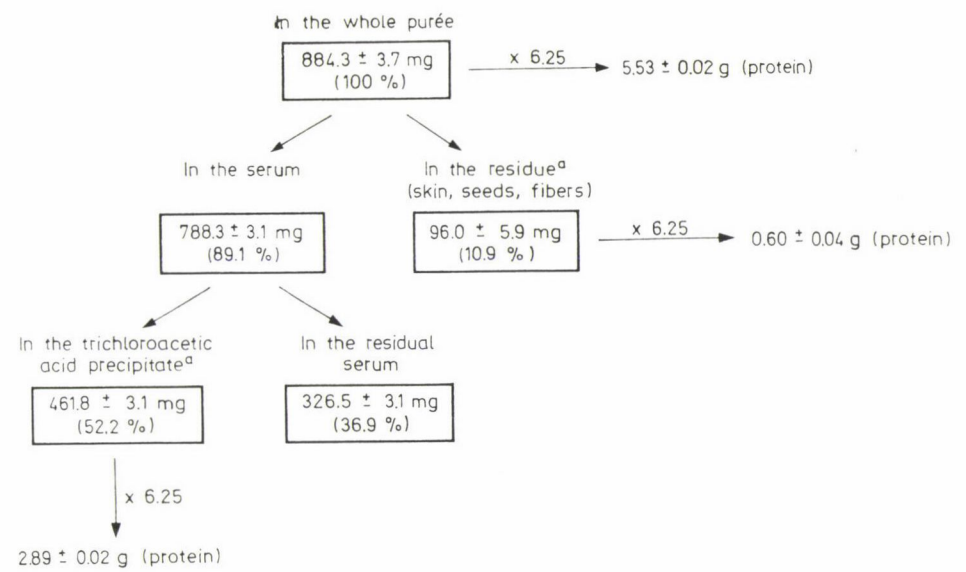


Fig. 2. Distribution of nitrogen content in 100 g purée made of the tomato cultivar "Mokka" as determined by Kjel-Foss analyzer. The data are mean values and standard deviations of three independent experiments.
[a] Calculated values

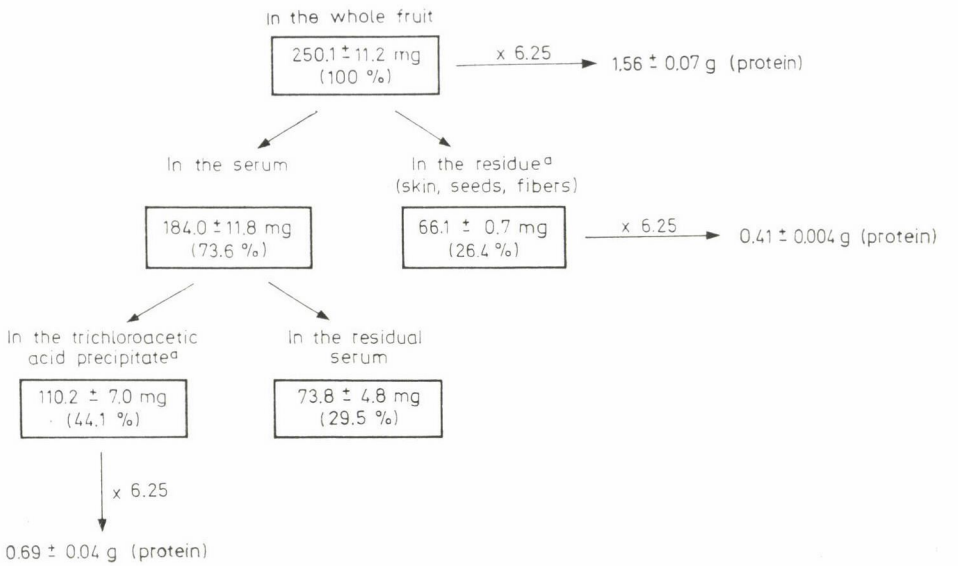


Fig. 3. Distribution of nitrogen content in 100 g fresh fruit of the tomato cultivar "K-549" as determined by Kjel-Foss analyzer. The data are mean values and standard deviations of three independent experiments.
[a] Calculated values

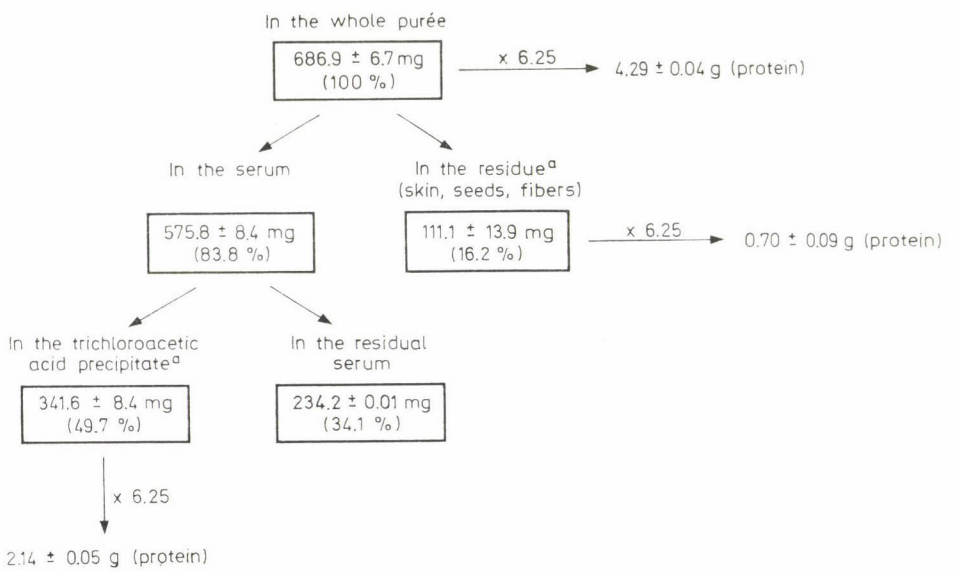


Fig. 4. Distribution of nitrogen content in 100 g purée made of the tomato cultivar "K-549" as determined by Kjel-Foss analyzer. The data are mean values and standard deviations of three independent experiments.
[a] Calculated values

According to material balances, about 26% of total nitrogen content was made up of compounds insoluble in serum (probably mainly proteins). About 44% of the soluble N-compounds could be precipitated with trichloroacetic acid. According to our earlier findings (KEVEI-PICHLER & PETRÓ-TURZA, 1974), however, this fraction contained — beside proteins — also peptides and nucleic acids, and thus gave no information about the actual water soluble protein content. It showed, on the other hand, that it was probably by similar investigations that ROWAN and co-workers (1958) came to the conclusion that 35–50% of the nitrogen content were made up of proteins. The fact that, in their review article DAVIES and HOBSON (1981) cite only the above reference in relation to this problem, indicates that similar investigations into tomato proteins have not been performed ever since.

Relating total N-content obtained in purées to solids content and comparing these values with those obtained for fresh tomatoes in a similar way shows the values measured in the concentrates of "Mokka" and "K-549" higher by 47.4 mg N and lower by 124.6 mg N, respectively, than those determined in the corresponding fresh tomatoes. The significant differences might be derived from the fact that purée is not manufactured from whole tomatoes but from a so-called tomato pulp from which skins, seeds and fiber substances have been removed, entirely or partially. The values might vary depending on the extent of removal.

The percentage ratio of soluble N-compounds increased in the purées as compared to fresh tomatoes, and the increase was somewhat higher in the purées of "Mokka" (from 71% to 89.1%) than in that of "K-549" (from 73.6% to 83.8%). This was to be expected, owing to heat-induced protein hydrolysis. At the same time the ratio of the N-compounds precipitable with trichloroacetic acid increased, too (for the "Mokka" purée from 44.4% to 52.2%, for the "K-549" purée from 44.1% to 49.1). This indicates that protein hydrolysis occurred only partially, to peptides.

2.2. Investigations with the amino acid analyzer

The qualitative and quantitative distribution of the free amino acids present in the samples was studied according to para, 1.2., with an amino acid analyzer.

The records showed in every case several minor peaks which could not be identified with any of the 20 amino acids applied as standards. These might correspond to peptides or other free amino acids.

In order to save time, the program was continued, in this case, only till the end of the elution of glutamine.

In order to detect also the peptides present in the samples, acid hydro-

Table 1

Amino acid composition of tomato cultivars "Mokka" and "K-549" before and after acid hydrolysis

	"Mokka"				"K-549"			
	before acid hydrolysis (mg per 100 g fresh weight)	before acid hydrolysis (mg N per 100 g fresh weight)	after acid hydrolysis (mg per 100 g fresh weight)	after acid hydrolysis (mg N per 100 g fresh weight)	before acid hydrolysis (mg per 100 g fresh weight)	before acid hydrolysis (mg N per 100 g fresh weight)	after acid hydrolysis (mg per 100 g fresh weight)	after acid hydrolysis (mg N per 100 g fresh weight)
Aspartic acid	151.30	15.92	414.46	43.60	67.82	7.13	158.28	16.66
Asparagine	51.68	10.96	—	—	39.54	8.38	—	—
Glutamic acid	468.85	44.64	1213.66	115.54	289.59	27.57	1103.85	105.09
Glutamine	275.71	52.85	—	—	185.96	35.64	—	—
Threonine	70.62	8.30	17.50	2.06	51.14	6.02	17.78	2.10
Serine	20.51	2.73	20.38	2.72	17.00	2.27	16.83	2.25
Glycine	2.27	0.42	9.89	1.85	3.27	0.61	8.58	1.60
Alanine	23.50	3.70	20.12	3.16	65.99	10.37	26.98	4.24
Cysteine	0.23	0.03	1.82	0.21	0.58	0.07	2.13	0.25
Valine	4.19	0.50	7.19	0.83	9.01	1.08	8.18	0.98
Methionine	1.67	0.16	—	—	2.37	0.22	0.79	0.07
Isoleucine	7.77	0.83	7.83	0.84	7.68	0.82	6.43	0.69
Leucine	6.48	0.69	9.20	0.98	6.29	0.68	6.87	0.73
Tyrosine	4.44	0.34	4.36	0.34	9.17	0.70	6.31	0.49
Phenylalanine	17.29	1.47	14.18	1.20	17.04	1.45	12.00	1.02
γ-aminobutyric acid	110.05	14.95	116.65	15.84	170.96	23.22	226.20	30.72
Lysine	12.90	2.47	15.21	2.91	14.31	2.74	12.96	2.49
Ammonia	1.50	1.23	42.36	34.84	1.83	1.50	18.66	15.34
Histidine	15.81	4.28	11.72	3.17	14.43	3.90	9.00	2.44
Arginine	10.15	3.26	8.80	2.83	10.28	3.30	7.54	2.43
Total	1256.92	169.73	1935.33	232.92	984.26	137.67	1649.37	189.59

The data are mean values of two parallels.

Table 2

Amino acid composition of purées made of tomato cultivars "Mokka" and "K-549" before and after acid hydrolysis

	"Mokka" purée				"K-549" purée			
	before acid hydrolysis (mg per 100 g purée)	before acid hydrolysis (mg N per 100 g purée)	after acid hydrolysis (mg per 100 g purée)	after acid hydrolysis (mg N per 100 g purée)	before acid hydrolysis (mg per 100 g purée)	before acid hydrolysis (mg N per 100 g purée)	after acid hydrolysis (mg per 100 g purée)	after acid hydrolysis (mg N per 100 g purée)
Aspartic acid	388.85	40.91	1263.88	132.96	348.73	36.69	595.35	62.63
Asparagine	212.11	44.97	—	—	133.42	28.29	—	—
Glutamic acid	1233.73	117.45	3995.46	380.37	1321.27	125.78	4300.44	409.40
Glutamine	372.57	71.42	—	—	104.19	19.97	—	—
Threonine	257.81	30.32	73.44	8.64	217.48	25.58	50.57	5.95
Serine	76.93	10.25	74.49	9.93	50.66	6.75	48.45	6.46
Glycine	4.41	0.82	44.81	8.36	3.56	0.66	35.26	6.58
Alanine	37.97	5.97	70.48	11.08	21.25	3.34	41.83	6.58
Cysteine	0.82	0.09	8.02	0.93	0.54	0.06	3.02	0.35
Valine	10.26	1.23	41.69	4.99	7.02	0.84	26.48	3.17
Methionine	3.91	0.37	6.38	0.60	3.47	0.33	—	—
Isoleucine	19.20	2.05	37.94	4.05	10.18	1.09	23.37	2.50
Leucine	16.57	1.77	51.71	5.52	9.86	1.05	33.95	3.63
Tyrosine	15.81	1.22	31.49	2.43	19.59	1.51	22.29	1.72
Phenylalanine	39.58	3.36	60.98	5.17	52.05	4.41	68.67	5.82
γ-aminobutyric acid	394.83	53.62	421.13	57.19	466.75	63.38	310.64	42.18
Lysine	33.57	6.43	76.83	14.72	26.12	5.00	52.64	10.09
Ammonia	67.66	55.65	173.74	142.90	53.00	43.59	82.33	67.72
Histidine	36.39	9.85	51.35	13.91	28.46	7.71	38.18	10.34
Arginine	24.29	7.81	47.34	15.22	19.63	6.31	33.14	10.66
Total	3247.27	465.56	6531.16	818.97	2897.23	382.34	5766.61	655.78

The data are mean values of two parallels.

lysis of the filtered sera as well as their subsequent analysis with the amino acid analyzer were carried out, too. Results are summarized in Tables 1 and 2.

Considering the experimental errors the given accuracy of tabulated data is exaggerated, notwithstanding, rounding was not performed as it would have resulted in still higher error when summing up values of different order of magnitude.

The amount of free amino acids was somewhat higher in the cultivar "Mokka" than in the cultivar "K-549". This can be explained by differences in solids content.

On the other hand, the distribution of free amino acids showed characteristic differences. Although glutamic acid and glutamine were dominating in both cultivars, the percentage distribution of the amino acids seemed to be more balanced in the traditional than in the new cultivar. With the exception of glutamic acid, glutamine and aspartic acid, the cultivar "K-549" contained higher amounts of all the amino acids than "Mokka".

Beside the differences, mentioned above considerable quantitative differences were observed for alanine and γ-aminobutyric acid. Following acid hydrolysis of the filtered sera — apart from the expected increments of aspartic and glutamic acids due to the hydrolysis of asparagine and glutamine — in the cultivar "Mokka" mainly the glutamic and aspartic acid contents increased, beside slighter increases in some other amino acids (glycine, valine, cysteine, leucine and lysine). Conversely, in the cultivar "K-549", practically only the γ-aminobutyric acid concentration increased beside glutamic and aspartic acids. The increments were obviously caused by hydrolysis of peptides whose composition appears to be different in the two cultivars.

Following hydrolysis, the amount of threonine considerably decreased in every sample. This indicates that in investigating free amino acids, the threonine peak might have been increased by peptides. This assumption was also supported by the corresponding chromatograms, where the peak of threonine was strongly distorted. Thus it was probable that from the amino acids threonine was present only in amounts smaller than those determined after hydrolysis. In later calculations this was not taken into account as the extent of peptide interference could have been estimated only.

The distribution of nitrogen content as obtained on the basis of amino acid analyzer studies is shown in Table 3.

Comparing the nitrogen content thus determined to the values obtained with the Kjel-Foss apparatus, deviations around $\pm 10\%$ were observed. Taking into account the error of the analytical methods, no significant difference was found between the values of total nitrogen content obtained by the two methods. This indicated that the amount of soluble proteins was so small that it remained within the limits of error of the measurements. This result confirmed our earlier statement according to which tomatoes contain soluble

Table 3

Distribution of the soluble N-content, on the basis of data obtained by amino acid analyser (mg per 100 g fresh fruit and purée, respectively)

Sample	Free amino acid N	Peptide N	NH_3	Total N	Total soluble N-content by Kjel-Foss analyser	Deviation of the total N-contents obtained by two different methods (%)
"Mokka" fruit	168.50 (72.4%)	63.19 (27.1%)	1.23 (0.5%)	232.92 (100%)	256.7	+10.2
"Mokka" purée	409.91 (50.0%)	353.41 (43.2%)	55.65 (6.8%)	818.97 (100%)	788.3	−3.7
"K-549" fruit	136.17 (71.8%)	51.92 (27.4%)	1.50 (0.8%)	189.59 (100%)	184.0	−2.9
"K-549" purée	338.75 (51.7%)	273.44 (41.7%)	43.59 (6.6%)	655.78 (100%)	575.8	−12.2

Table 4

Quantitative distribution of free amino acids and peptides

Sample	Soluble solids (g per 100 g)	Free amino acids	Peptides	Total
		(g per 100 g soluble solids)		
"Mokka" fruit	7.49±0.03	16.8	8.5	25.3
"Mokka" purée	23.87±0.07	13.3	13.3	26.6
"K-549" fruit	5.70±0.04	17.2	11.4	28.6
"K-549" purée	20.37±0.09	14.0	14.0	28.0

proteins in minute amounts only (KEVEI-PICHLER & PETRÓ-TURZA, 1974). However, the good agreement between the results obtained with two measurements, independent from each other, confirmed also that errors of measurements have not been committed in the course of investigations.

The free amino acid and peptide contents of the samples as expressed in g per 100 g soluble solids are shown in Table 4.

According to the Table, free amino acid concentrations in fresh tomatoes were higher than those of peptides. In the cultivar "Mokka" the ratio of the two kinds of compounds approximates 2 : 1 while in the cultivar "K-549" it is only 1.5 : 1. During evaporation, this ratio changed in both cultivars to 1 : 1. During concentrate manufacture the amount of free amino acids decreased, on the average, by 20% while that of the peptides increased as a result of partial protein hydrolysis. The decrease in insoluble protein content (nearly 60% for the cultivar "Mokka" and about 40% for the cultivar "K-549") was clearly shown also by the nitrogen balances.

In the tomato cultivars investigated the amino acids accounted for a considerable part of total solids, i. e. in the cultivar "Mokka" for 11.2% (total solids: 11.25 g per 100 g), in the cultivar "K-549" for 11.9% (total solids: 8.24 g per 100 g). These data considerably disagree with those published by DAVIES and HOBSON (1981) according to which free amino acids account, on the average, only for about 2.5% of total solids of tomatoes. Moreover, there was a considerable difference between the protein contents given by these authors (8%, on the average) and those measured by us i. e., our results showed that mainly insoluble proteins are present in tomatoes whose concentrations (applying the factor 6.25) made up only 5% of total solids in both cultivars. It is, however, probable that for calculating the protein content of tomatoes from the N-content, the multiplication factor of 6.25 is not suited as — similarly to the peptides and free amino acids — they presumably contain much glutamic acid and aspartic acid. The multiplication factors valid for these are, however, much higher (Glu = N×10.5; Asp = N×9.5). If we assume the multiplication factor — taking into account the data obtained after peptide hydrolysis — to be about 9, the actual protein content measured by us only approximates the data of the literature but does not reach them, notwithstanding the fact that — according to the solids content higher in our samples than the values published by DAVIES and HOBSON (1981) — they ought to have surpassed the latter.

As to the occurrence of peptides in tomatoes, no hint whatsoever at their existence was found in the literature, in spite of the fact that their presence seems to be fairly obvious. Our measurements proved their amounts not to be negligible at any rate. Finally, the total amount of free amino acids and peptides accounted for about 26–29% of soluble solids in the samples. When comparing this to the amount of 11–13% of organic acids, it seems highly probable that both amino acids and peptides participate in the formation of the taste of tomatoes. The question that remains is only, in which direction they influence it. The answer to this question was sought by us by means of sensory analyses whose results are going to be published in Part III of this series of papers, in the journal "Die Nahrung".

*

The authors thank Ms E. GODEK-KEREK for determining the nitrogen content of the samples, further Mr J. FEHÉR for conscientious performance of the investigations carried out with the amino acid analyser.

Literature

DAVIES, J. N. & HOBSON, G. E. (1981): The constituents of tomato fruit — the influence of environment, nutrition, and genotype. *CRC Crit. Rev. Fd Sci. Nutr., 15*, 205–280.

KEVEI-PICHLER, E. & PETRÓ-TURZA, M. (1974): *Vizsgálati módszerek kidolgozása egyes élelmiszerek minősítésére az íz- és illatanyagok (aroma) meghatározása alapján.* (De-

velopment of methods for qualifying certain food products on the basis of determination of flavour substances.) Research Report. Central Food Research Institute, Budapest, pp. 5–8.

PETRÓ-TURZA, M. & KEVEI-PICHLER, E. (1975): *Vizsgálati módszerek kidolgozása egyes élelmiszerek minősítésére az íz- és illatanyagok (aroma) meghatározása alapján.* (Development of methods for qualifying certain food products on the basis of determination of flavour substances.) Research Report. Central Food Research Institute, Budapest pp. 5–7.

PETRÓ-TURZA, M. (1986—1987): Flavor of tomato and tomato products *Fd Revs Int., 2,* 309–351.

PETRÓ-TURZA, M., SZÁRFÖLDI-SZALMA, I., FÜZESI-KARDOS, K. & HAJDÚ, F. (1989): Study on taste substances of tomato. — Part I. Analytical investigations into the sugar, organic acid and mineral contents. *Acta Alimentaria, 18,* 97–106.

ROWAN, K. S., PRATT, H. K. & ROBERTSON, R. N. (1958): The relationship of high-energy phosphate content, protein synthesis, and the climacteric rise in the respiration of ripening avocado and tomato fruits. *Aust. J. biol. Sci., 11,* 329. — ref: DAVIES & HOBSON (1981).

BOOK REVIEW

Food structure — Its creation and evaluation

J· M. V. BLANSHARD & J. R. MITCHELL (Eds)

Butterworths Borough Green, Sevenoaks, Kent, England, 1988, 504 pages

The book contains the proceedings of the 44th Nottingham Easter School in Agriculture Science Symposium held in the subject with international participation. The 26 lectures deal with nearly all problems of the topic. On one hand it endeavours to answer the problems "How can we characterize the texture of foodstuffs in a manner that will reflect human perception", and on the other hand the question "how may we preserve, formulate and process products in a way that will be acceptable to the consumer?"

In the light of the questions the book investigates firstly the role of ingredients in building food structures, and secondly the contribution and function of the ingredients within that structure. Obviously, due to the character of the problem final solution cannot be found. However, the reader will find not only state of the art reviews of molecular interactions in homogeneous and heterogeneous multicomponent systems, but also reviews of the macroscopic physics of their mechanical properties of the more phenomenological approach necessary as systems increase in complexity and, finally, of the variety of techniques and strategies necessary to evaluate their properties if they are to be acceptable to the consumer.

Presentations in the book may be divided into two parts. The first part of the book considers fundamental concepts and discusses dispersed food systems such as gels, emulsions and foams together with the physical and chemical properties of food polymers. It goes on to look at structured food systems such as fibrous and non-fibrous systems and concludes with an evaluation of food structure by microscopic, rheologic and sensory means.

The wide range and ample literary references supplement the lectures in the book. A 15 pages index makes the handling and processing of the information easier. The preface and the list of participants complete the content.

Workers in the food industry as well as in universities and research institutes will find this book a good summary of current food structure research.

I. VARSÁNYI

CATERING & HEALTH

International Journal of Hygiene and Nutrition in Catering (Foodservice)

Edited by R. J. L. Allen
Honorary President
British Dietetic Association

and

R. H. G. Charles
Adviser, World Health Organisation

The International Journal of Catering and Health has been established to provide a forum for research papers and review articles, written by experts in all areas concerned with the nutritional and hygienic aspects of catering (foodservice). The Journal includes within its scope current hygienic problems in institutional and airline catering; nutritional, microbiological, toxicological and epidemiological and educational aspects of catering; health aspects of new technology and legislation; pest control, and catering in hot climates.

Also included are book reviews and reports of relevant meetings of national and international organisations, such as WHO working party on health aspects of catering.

Notes for contributors are available from Dr R. H. G. Charles, 54 Chatsworth Road, London W5 3DB, England.

Subscriptions rates per volume of 4 issues, postpaid: £ 55/US $ 77, A B Academic Publishers, P. O. Box 97, Berkhamsted, Herts HP4 2PX, England.

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

RECENTLY ACCEPTED PAPERS

Investigation into the interaction between sensory properties in meat products
URBÁNYI, GY. & PÁLFI, ZS.

Comparison of rheological, chemical and sensory properties of pulped tomatoes preserved by various techniques
JIMENEZ, L., FERRER, J. L. & CHICA, A.

Insect damage to wheat grain
LORENZ, K.

NOTICE TO CONTRIBUTORS

General. Manuscripts in English or Hungarian should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

Title. The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

Summary. The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

Text. The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

Symbols. Units, symbols and abbreviations should be used according to SI (System International) rules. Unusual abbreviations must be explained in the text or in an appendix.

Figures and tables. Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

References. The reference list (Literature) should be in alphabetical order as follows:

Periodicals: Names and initials of all the authors; year of publication in parentheses; colen; title of the paper; title of the periodical; inclusive page numbers.

Books: Names and initials of all the authors; the year of publication in parentheses; colon; title of the book; publishing firm, place of publication; inclusive page numbers.

Detailed instructions for publishing in Acta Alimentaria are available from the Editorial Office.

Authors will receive one set of proofs which must be corrected and returned at the earliest convenience to the Editorial Office. In this phase major alterations of the text cannot be accepted.

Offprints. We supply 100 offprints free of charge. Additional copies can be ordered.

No page charges are levied on authors or their institutions.

Acta Alimentaria is surveyed by Current Contents/Agriculture, Biology and Environmental Sciences, ASCA, BIOSIS, Nutrition Abstracts and Reviews.

ACTA ALIMENTARIA

VOLUME 18 No. 1 — 1989

CONTENTS

Index: 23.069

ACTA

ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 18 **June 1989** **Number 2**

Akadémiai Kiadó
Budapest

ISSN 0139—3006

CODEN ACALDI

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

Distributor:

KULTURA, Hungarian Foreign Trading Company
P.O. Box 149, H-1389 Budapest 62, Hungary

Publication programme, 1989: Volume 18 (4 issues)
Subscription prices per volume: US $ 130,00 plus 15% postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 18

1989

AKADÉMIAI KIADÓ
BUDAPEST

Acta Alimentaria, Vol. 18 (2), pp. 121—125 (1989)

CARBOHYDRATE, PROTEIN AND TRACE ELEMENT CONTENT OF *PHALARIS CANARIENSIS* AND *ECHINOCHLOA CRUSGALLI* GRAINS

K. M. KANDEEL,[a] G. A. YACOUT,[a] M. M. EL SADEK[b] and A. A. ZAKY[a]

[a]Department of Biochemistry, Faculty of Science, Alexandria University, Alexandria. Egypt
[b]Department of Chemistry, Faculty of Science, Alexandria University, Alexandria. Egypt

(Received: 27 March 1987; accepted: 6 September 1988)

Phalaris canariensis (I) and *Echinochloa crusgalli* (II) grains were investigated, regarding to carbohydrate, protein and trace element content. It was found from the obtained results that the two examined grains gave significantly different results, whereas, *Phalaris canariensis* grains had a high content of proteins; *Echinochloa crusgalli* grains were rich in carbohydrate as well as trace elements.

Keywords: carbohydrate, protein, trace element, Gramineae

Phalaris canariensis and *Echinochloa crusgalli* are annual plants belonging to the family of Gramineae, which is used as human food (Japanese millet), birds' feed as well as forage for animals. These grains were subjected to several studies including fatty acids, and sterols contents (MALIC & WILLIAMS, 1966; BOWDEN & WILLIAMS, 1971; KANDEEL et al., 1987).

The aim of this work was to analyse these grains for carbohydrate, protein and trace elements contents due to the importance of these components in the nutritional aspects.

1. Materials and methods

1.1. Extraction of proteins and carbohydrates

Finely ground grains of I and II (1.5 kg of each) were refatted separately by successive soaking according to AMIN and KANDEEL (1972), in acetone, ether and methanol. The dried residue of each was boiled in succession, separately with distilled water (3 dm^3), 1.5% $NaHCO_3$ (4 dm^3), and 4% NaOH solution (4 dm^3), for three hours in each solvent, respectively. Each extract was centrifuged off and the obtained clear supernatant of each extract was acidified with 50% acetic acid solution to pH 6.5 followed by centrifugation to separate the protein fraction; the carbohydrate fraction of each extract was precipitated with ethanol (3 : 1, v/v). The obtained carbohydrate fractions of grains I and II were washed separately with ethanol, acetone and ether, respectively, then dried (Table 1).

Akadémiai Kiadó, Budapest

1.2. Iodine test for the carbohydrate fractions

Dilute iodine solution (0.01%) used to examine the obtained carbohydrate fractions from grains I and II, gave a blue colour for each.

Table 1

Carbohydrate and protein content (% on dry weight) of different solvent extracts of grains I and II

Fraction	I	II
Carbohydrate		
water extract	0.743	1.274
1.5% $NaHCO_3$ extract	1.144	1.300
4% NaOH extract	0.722	0.900
total	2.609	3.474
Protein		
water extract	—	—
1.5% $NaHCO_3$ extract	—	—
4% NaOH extract	5.370	1.370

Table 2

Amino acid compositions of the protein hydrolysates of I and II grains

Amino acid	I	II
	(concentrate g per 100 g protein hydrolyzate)	
Aspartic	0.548	0.283
Threonine	0.126	0.078
Serine	0.169	0.076
Glutamic	2.571	0.921
Proline	0.400	0.100
Glycine	0.283	0.131
Alanine	0.379	0.187
Valine	1.799	0.218
Methionine	0.140	0.023
Isoleucine	0.097	0.025
Leucine	0.944	0.304
Tyrosine	0.081	0.044
Phenylalanine	0.323	0.120
Histidine	0.075	0.037
Lysine	0.104	0.070
Arginine	—	0.061

1.3. Chromatographic examination of the carbohydrate fractions

A sample of 10 mg of each carbohydrate fraction was hydrolysed with sulfuric acid solution (4 cm^3, 1 N) at 100 °C for 17 hours. Each hydrolysate was neutralized separately and examined by paper chromatography, on Whatman No. 1 filter paper, using butanol–ethanol–water (5 : 1 : 4, v/v) solvent system and aniline phthalate (2%) as spray reagent. Authentic samples of galactose, glucose, xylose and arabinose were used as standards. The chromatogram of each fraction from grains I and II revealed one spot corresponding to glucose.

1.4. Total protein content

The total protein contents extracted by 4% NaOH of grains I and II were determined, separately according to Lowry's modified method (HARTREE, 1972). Solutions of bovine serum albumin (0.1 up to 0.8 mg cm^{-3}) were used as standards. Yield: 15% and 4.2% for grains I and II, respectively.

1.5. Analysis of the protein hydrolysates

Samples (1 g each) from the obtained protein fractions of grains I and II were hydrolyzed separately according to HOSAKU & YOSHIAKI, 1979.

Each hydrolyzate was examined using Beckman 118 CL amino acid analyzer (Table 2).

1.6. Trace elements

Portions (10 g each) from grains I and II were ashed separately (ARNOLD et al., 1981) in carbolite oven at 500 °C (±50 °C). Each obtained ash was completely digested by gentle refluxing with conc. nitric acid, followed by filtration and diluted to 50 cm^3 with deionized water. Ash yields for grains I and II were 5.5 and 6.0%, respectively.

1.7. Atomic absorption measurements

Each obtained solution was analysed separately for the presence of Cd, Cr, Mn, Cu, Ni, Sn and Fe at 228.8, 357.9, 279.5, 324.7, 232.0, 224.6, 248.3 nm, respectively, using a Jarrel Ash Model 850 Atomic Absorption Double Beam Spectrophotometer with an air-acetylene flame burner and a hydrogen lamp for background correction. The instrument was equipped with completely computer controlled electronics. Atomic absorption calibration standards were prepared with BDH standards (BDH Chemical Ltd., Poole, England) (Tables 3 and 4).

2. Results and discussion

The carbohydrate fractions obtained from grains I and II, extracted by distilled water, 1.5% $NaHCO_3$, and 4% NaOH, were 2.609 and 3.474 wt %, respectively.

Examination of these fractions by dilute iodine solution, showed that these were composed of starch; the result was confirmed by acid hydrolysis and paper-chromatographic analysis of the neutralized hydrolysate of each fraction.

Table 3

Calibration curves of trace elements

Metal	A	C	A	C	A	C	A	C
Cd	32	0.3	93	1	138	1.5	235	2.5
Cr	12	1	56	5	81	7	120	10
Mn	25	0.5	49	1	146	3	250	5
Ni	40	2	80	4	158	8	230	12
Sn	1	10	6	50	10	75	13	100
Fe	2	0.5	10	2.5	20	3	30	7.5
Cu	68	0.5	272	2	535	4	910	7

A : absorbance C ; concentration ($\mu g\ cm^{-3}$)

Table 4

Comparative analytical data for trace element content of grains I and II (mg per 100 g dry weight)

Sample	Cd	Cr	Mn	Cu	Ni	Sn	Fe
I	0.015	0.408	3.315	0.39	0.357	9.2	29.0
II	0.023	1.15	3.246	1.63	0.55	166.08	62.0

On the other hand, only one protein fraction was extracted from each grains I and II by 4% NaOH solution. The yield was 5.37 and 1.37 wt %, respectively. It was remarkable that no proteins were separated from water or 1.5% $NaHCO_3$ extracts, although repeated several times. The total protein content of each separated fraction was found to be 15.0 and 4.2 wt % for grains I and II, respectively, indicating that most of these proteins were conjugated.

Furthermore, the compositions of the only identified amino acids of the extracted protein hydrolysates of grains I and II were investigated using the amino acid analyzer, and it was noticed from the obtained data that

grain I was richer in the essential amino acids: threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine than grain II.

In addition, ashing of both kinds of grains revealed about 5.5 and 6.0 wt % ash for grains I and II, respectively. Analysis of the obtained ashes, separately using atomic absorption spectrophotometer, showed that grain I was rich in Cd, Cr, Cu, Ni and Fe content, compared with the amount of these elements in grain I with the exception of Mn. It was also remarkable that a relatively too high Sn content was detected in grain II in comparison to grain I.

In spite of the differences in the obtained results, it appears that, both grains I and II have high nutritional value.

3. Conclusions

— *Phalaris canariensis* grains have higher protein and essential amino acid content than *Echinochloa crusgalli* grains.

— *Echinochloa crusgalli* grains have more starch and trace elements content than *Phalaris canariensis* grains.

— *Echinochloa crusgalli* grains have a high content of Sn more than the normal value.

Literature

AMIN, EL S. & KANDEEL, K. M. (1972): The hemicellulose of pollen grains of *Phoenix dactylifera*. *Carbohydr. Res.*, *25*, 261–268.

ARNOLD, E., JOSEPH, J. & DAVID, J. (1981): *Standard methods for the examination of water and waste water in atomic absorption method.* American Publishers Health Association, Washington, pp. 143–152.

BOWDEN, N. B. & WILLIAMS, M. P. (1971): Sterols in grass seeds. *Phytochemistry*, *10*, 3135–3137.

HARTREE, E. F. (1972): A modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.*, *48*, 422–425.

HOSAKU, K. & YOSHIAKI, M. (1979): Gas-liquid-chromatographic determination of amino acids and vertical distribution of proteinaceous substances in sea water. *J. Oceanograph. Soc., Japan*, *27*, 27–30.

KANDEEL, K. K., YACOUT, G. A., EL SADEK, M. M. & ZAKY, A. A. (1987): Comparative study of fatty acids and sterols of lipids of *Phalaris canariensis* and *Echinochloa crusgalli* grains. *Bull. Fac. Sci. Alexandria Univ.*, *28*, 21–26.

MALIC, M. Y. & WILLIAMS, W. D. (1966): Composition of the seed oil of *Phalaris canariensis*. *J. Sci. Fd Agric.*, *17*, 174–175.

Acta Alimentaria, Vol. 18 (2), pp. 127—141 (1989)

INVESTIGATION INTO THE INTERACTION BETWEEN SENSORY PROPERTIES IN MEAT PRODUCTS

I. RELATIONSHIP BETWEEN COLOUR AND TASTE SENSATION AND COLOUR AS MEASURED OBJECTIVELY AND SENSORILY OBSERVED

GY. URBÁNYI and ZS. PÁLFI

Department of Food and Refrigerating Technology, University of Horticulture and Food Industry,
H-1118 Budapest, Ménesi út 43–45. Hungary

(Received: 4 December 1987; accepted: 11 August 1988)

Experiences gained in sensory evaluations have shown panel members to be influenced by other properties in judging certain characteristics. Particularly interesting can be the effect of colour. In earlier experiments this mutual effects were studied in foods of plant origin. Since there are few observations available on this interplay in relation to foods of animal origin, we decided to study this problem.

Meat products of homogeneous consistency were selected the colour of which could be affected with food colouring agents without causing changes in other properties. Evaluation were carried out with panels of experienced and inexperienced judges. Control evaluations were carried out in the dark and also under red light.

In addition to sensory evaluation the colours of the samples were measured instrumentally as well and the relationship between sensory values and measured characteristics was established.

In the course of scoring the taste values obtained were affected by the colour differing from the natural. This effect was more intensive with inexperienced panel members than with the experienced ones. Thus, it seems expedient to compose panels from highly experienced members or take care to train properly the colleagues asked to participate in sensory evaluations. More objective judgement of the taste of foods can be improved by applying conditions impeding the comparison of colours. For instance by judging in the dark or in light covering differences in colour or causing all the samples to have a uniform colour. In cases like this colour scoring has to be done under different circumstances.

In sensory evaluation of colour, in most cases, there was a correlation between the number of scores and value "a*" characterising red in the CIELAB system. Correlation was found also between the sensory value of colour and "L*" values.

The above experiences show that it is useful to carry out instrumental colour determination simultaneously with the sensory evaluation because the former supports the judgement of panel members and the threshold values as determined by sensory evaluation of the colour characters may be useful in the classification of the product.

Keywords: effect of colour, meat products, sensory tests, sensory properties

Experiences gained in sensory evaluations have shown that panel members may be influenced in their judgement on certain properties by other characteristics. The influence of appearence, particularly that of colour may

be of importance because it permits to draw conclusions as to the state of the given food product (ripeness, spoilage, etc.). This shows that colour has a psychological as well as physiological effect upon human judgement. KOSTYLA and CLYDESDALE (1978) describe the Wheately experiment, in the course of which a meal consisting of meat, peas and baked potatoes was served, illuminated with coloured light and from time to time with natural light, too. Panel members complained of acute nausea caused by the unnatural illumination of meat with blue, peas with red and potatoes with green light.

Already in the first half of this century a study was made on the sensory evaluation of jelly (MOIR, 1936) in the course of which the judges made more frequently a mistake when samples had an unnatural colour. PANGBORN and co-workers (1963) made similar observations in relation to evaluating sweet taste.

AMERINE and co-workers (1965) found that when otherwise of the same taste and odour, margarine was tinted white and yellow the white samples could be sold only at a lower price.

ELBE and JOHNSON (1971) found greater differences in the scores of green peas and MAGA (1973) in that of potatoes if samples had different colours. The experiment of the latter author was repeated with blindfolded panel members and differences were not observed in the taste of the samples.

On the basis of further experiments MAGA (1974) concluded that the meaning of certain fundamental taste sensations may be changed by psychological colour associations. According to these observations green colour was found to intensify the sensation of sweet, while yellow reduced it and red did not exert any effect. The sensation of sour was modified by green and yellow but they did not affect bitter taste. It is interesting to note that the sensation of salty was not affected significantly by variation of colour.

Aroma and flavour of a variety of tinted foodstuffs was tested by a panel in a study of CHRISTENSEN (1985). With samples of natural colour panel members found aroma and flavour more intense and better. Only the aroma of the sample was found to be affected significantly by colour. If samples were tested blindfolded differences were not observable.

Experiments carried out with ice-cream showed (ANON., 1980) that the aroma of the samples could be identified only if samples were of characteristic colour.

On testing meat products BOOMANN and EBERBACH (1976) found that the judgement of products was influenced by the colour of illumination.

HOOD (1986) observed that if the colour of marketed meat was affected by the wrapping difficulties arouse in selling it.

In earlier experiments the author (URBÁNYI, 1982) investigated the effect of colour upon taste in the case of food products of vegetable origin. Since the same problem arises pertinent to meat products and very little

related reference was found in the literature it was decided to study the problem.

Since the requirement to compare sensory values to instrumental measurements of the properties of foods arises more and more frequently (KOSTYLA & CLYDESDALE, 1978; HEATH, 1985) it was deemed necessary to investigate this problem, as well. In related literature reference was found mostly on consistency (MOSKOWITZ et al., 1979; SAWYER et al., 1984; MONTEJANO et al., 1985). Relationship between the colour parameters of foods in the Hunter system and various sensory qualities or instrumentally measurable properties were studied and certain correlations established by MCLELLAN and co-workers (1984).

Correlations between sensorily obtained scores and the instrumentally measurable colour characteristics were evaluated by mathematical statistical methods.

1. Materials and methods

1.1. Materials

In selecting materials for sensory tests samples of homogeneous consistency were used. The samples were tinted with food colouring agents which did not influence other properties of the foods. The samples were taken from the market and consisted in various kinds of fresh sausages, and canned ham.

To achieve the desired tinted colour the following permitted food colouring agents, devoid of a teste of their own, were used:

- neucoccin (red);
- rum brown (mixture of neucoccin, tartrazin, indigo carmine, brilliant black);
- tartrazin (yellow) and indigo carmine (blue) mixed to give a green colour.

To tint the fresh sausage ("pariser") it was sliced and the slices were submerged for 1 minute in the following solutions:

neucoccin	0.05 and 0.01%
rum brown	0.07 and 0.03%
green (tartrazin–indigo carmine)	0.005 and 0.0025%.

The concentration of the dye was determined in preliminary experiments and care was taken to achieve a non too unnatural colour. The red tint suggests the use of more spice, the brownish a stronger smoking and the greenish an early stage of spoilage. The control sample was submerged in plain water for an identical period.

Canned meat was mashed and the dye solutions were admixed to obtain the desired colour. To a sample of 150 g 15 cm^3 of the following solutions was added:

neucoccin	0.25 and 0.125%
rum brown	1.00 and 0.50%
green (tartrazin–indigo carmine)	0.0375 and 0.01%

1.2. Methods

1.2.1. Sensory evaluation. The colour and taste of all the samples was tested by scoring, maximum score value being 9 for each parameter. Panels consisted of 8 to 13 members. Re-tasting of samples was permitted. One panel consisted of well trained, highly experienced members and the second panel from inexperienced, untrained panelists from students.

Table 1

First sensory test results of "pariser" slices
(KRAMER, 1963)

Sample	In light				In darkness	
	colour		taste		taste	
	sum of the rank serial number	numerical order	sum of the rank serial number	numerical order	sum of the rank serial number	numerical order
Trained panel (n = 8)						
Natural colour	25.5	3	30.0	5	32.5	4
red: 0.1%	30.0	4	26.5	2	24.0	1
0.05%	25.0	2	28.5	4	25.5	2
green: 0.025%	39.0	6	40.5	6	38.5	6
0.05%	54.5**	7	49.0**	7	28.5	3
rum brown: 0.03%	19.0	1	22.0	1	39.5	7
0.07%	31.0	5	27.5	3	35.5	5
Untrained panel (n = 10)						
Natural colour	41.0	5	40.5	4	33.0	1.5
red: 0.01%	23.5*	1	36.0	3	38.0	4
0.05%	30.5	3	26.0	1	33.0	1.5
green: 0.0025%	53.0	6	45.5	5	47.0	6
0.005%	57.0*	7	51.0	7	43.5	5
rum brown: 0.03%	25.0	2	35.0	2	37.5	3
0.07%	40.0	4	46.0	6	48.0	7

P-values (n = 8) *: Significant at 95% probability level (18–46)
**: Highly significant at 99% probability level (16–48)
(n = 10) *: Significant at 95% probability level (25–55)
**: Highly significant at 99% probability level (22–58)

Table 2

Second sensory test results of "pariser" slices
(KRAMER, 1963)

Sample	In light				In darkness	
	colour		taste		taste	
	sum of the rank serial number	numerical order	sum of the rank serial number	numerical order	sum of the rank serial number	numerical order
Trained panel (n = 8)						
Natural colour	25.0	3.5	33.5	5	33.5	6
red: 0.01%	17.0*	1	26.0	3	50.5**	7
0.05%	33.5	5	29.0	4	27.0	2
green: 0.0025%	49.5**	6	45.0	7	29.0	4.5
0.005%	50.5**	7	44.0	6	26.5	1
rum brown: 0.03%	23.5	2	23.5	2	28.5	3
0.07%	25.0	3.5	23.0	1	29.0	4.5
Untrained panel (n = 9)						
Natural colour	34.0	4	38.5	5	29.0	1
red: 0.01%	15.0**	1	28.5	1	37.5	5
0.05%	31.0	3	29.5	2	36.0	4
green: 0.0025%	60.0**	7	36.5	4	38.5	6
0.005%	56.0**	6	48.0*	7	34.5	3
rum brown: 0.03%	19.5*	2	40.5	6	45.5	7
0.07%	36.5	5	30.5	3	32.0	2

P-values (n = 8) *: Significant at 95% probability level (18–46)
**: Highly significant at 99% probability level (16–48)
(n = 9) *: Significant at 95% probability level (22–50)
**: Highly significant at 99% probability level (19–53)

As a control the samples were tested also in a dark room with red illumination. The red light covered the colour differences, thus, these could not influence the judgement of taste.

Results were evaluated according to KRAMER (1963), although this does not give information on the differences among samples in case of several significantly differing samples (JOANES, 1985).

1.2.2. Instrumental colour measurement. Objective colour determinations were carried out with Momcolor-D tristimulus instrument by the reflection method. The white etalon of the National Office of Measures, Hungary, No. 80–26–00 was used as reference, with the following components: X_1-64.90, X_2–15.82, Y-82.91, Z-95.14.

Geometry of the measuring head 0°/45°, with an optic angle of 2° and CIE C illumination. Diameter of the diaphragm 5 mm.

Two parallel series of measurements were carried out. The sensory evaluations were carried out by two different panels.

2. Results

2.1. Correlations between sensory characteristics

The first test material, tinted slices of the fresh sausage called "pariser", were tested by two different panels at two different points of time (experienced and inexperienced panel members). The results of these tests were evaluated according to Kramer and are shown in Tables 1 and 2.

Table 3

Values of correlation coefficient between the sensory properties of "pariser" slices (n = 7)

Properties compared	Correlation coefficient (r)	
	trained panel	untrained panel
1st test		
Colour — taste (in light)	0.834*	0.918**
Colour — taste (in the dark)	0.300	0.512
Taste (in light) — taste (in the dark)	0.417	0.812*
2nd test		
Colour — taste (in light)	0.581	0.928**
Colour — taste (in the dark)	0.208	0.025
Taste (in light) — taste (in the dark)	0.104	0.163

* Significant at $P \geq 95\%$ probability level ($r = 0.754$)
** Highly significant at $P \geq 99\%$ probability level ($r = 0.874$)

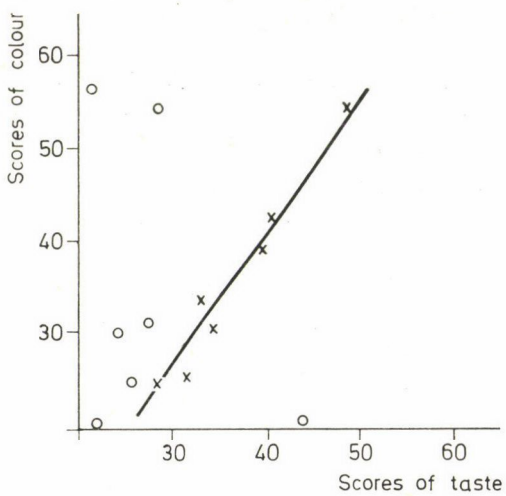


Fig. 1. Relationship between sensory taste and colour scores based on summarized scores of seven investigations received by seven panelists. (Trained panel, 1st test) x: colour — taste (in light) (r = 0.834) o: colour — taste (in darkness)

Table 4

Sensory test results of canned "veronese"
(KRAMER, 1963)

Sample	In light				In the dark	
	colour		taste		taste	
	sum of the rank serial number	numerical order	sum of the rank serial number	numerical order	sum of the rank serial number	numerical order
Trained panel (n = 9)						
Natural colour	26.5	3	28.5	1	39.5	6
red: 0.125%	23.5	2	42.0	7	31.0	2
0.25%	23.0	1	36.0	4	30.0	1
green: 0.01%	41.0	5	39.0	6	35.5	4
0.0375%	55.0**	7	38.0	5	42.5	7
rum brown: 0.5%	39.0	4	33.5	2	34.5	3
1.0%	44.0	6	35.0	3	39.0	5
Untrained panel (n = 13)						
Natural colour	40.0	3	44.0	1	44.0	1
red: 0.125%	25.0**	1	50.0	3	52.5	5
0.25%	36.0	2	45.5	2	50.0	2
green: 0.01%	72.0*	6	61.5	7	52.0	4
0.0375%	76.5**	7	55.5	6	59.0	7
rum brown: 0.5%	52.0	4	54.5	5	55.5	6
1.0%	62.5	5	53.0	4	50.0	3

P-values (n = 9) *: Significant at 95% probability level (22–50)
**:Highly significant at 99% probability level (19–53)
(n = 13) *:Significant at 95% probability level (35–69)
**:Highly significant at 99% probability level (31–73)

Results of colour and taste show, in several cases, correspondence. Particularly in the case of samples ranked "good" or "bad". Significantly differing taste scores belong always to significantly differing colour scores.

In taste tests, carried out in the dark, corresponding results of the two panels are rarely found. Significant difference was found only in a single case. The taste of this sample, when judged in the dark, was given the lowest score at the 99% probability level, while judged in light had the highest score at 95% probability level.

To establish the correlations among sensory values linear regression analysis was applied to the summarized scores. The results are given in Table 3. The correlation between taste and colour values scored by the highly experienced panel is illustrated in Fig. 1.

The correlation was found significant between colour and taste, when scored in light (except for the 2nd test of the trained panel). Significant cor-

Table 5

Sensory test results of canned ham
(KRAMER, 1963)

Sample	In light				In the dark	
	colour		taste		taste	
	sum of the rank serial number	numerical order	sum of the rank serial number	numerical order	sum of the rank serial number	numerical order
Trained panel (n = 9)						
Natural colour	34.5	4	33.0	2	30.5	2
red: 0.125%	22.5	2	30.0	1	35.0	4.5
0.25%	14.5**	1	35.5	4	35.0	4.5
green: 0.01%	49.0	6	34.0	3	49.0	7
0.375%	60.0	7	45.5	7	34.0	3
rum brown: 0.5%	31.5	3	36.5	5	39.0	6
1.0%	40.0	5	37.5	6	29.5	1
Untrained panel (n = 8)						
Natural colour	24.5	3	31.0	3	35.0	5
red: 0.125%	20.5	1	32.0	4	31.0	3
0.25%	21.0	2	28.5	1	36.5	6
green: 0.01%	41.0	6	34.5	6	37.0	7
0.0375%	56.0**	7	32.5	5	31.5	4
rum brown: 0.5%	29.0	4	28.5	2	26.0	1
1.0%	32.0	5	37.0	7	27.0	2

P-values (n = 8) *: Significant at 95% probability level (18–46)
**: Highly significant at 99% probability level (16–48)
(n = 9) *: Significant 95% probability level (22–50)
**: Highly significant at 99% probability level (19–53)

relation was not found between colour and taste, when tested in the dark and only on a single occasion between taste judged in light and in darkness. The above findings show that the colour influenced the judgement on taste. The judgement of the untrained panel was more affected by colour. The lack of correlation between the two tests of taste show that there were no real differences between the tastes of the samples.

The next subject of study were the mashed meat samples of "veronese" and canned ham. The results of the tests are given in Tables 4 and 5.

The results resemble those obtained with the first material, e.g. with the "pariser". However, in the case of these two materials less correspondence was found between colour and taste, particularly in the values as given by the trained panel. It is interesting to note that the lowest values were assigned to the samples tinted green. Since the greenish colour is characteristic of spoilage this moved panel members to score these samples very low.

Table 6

Values of correlation coefficients between the sensory properties of mashed canned meats

Properties compared	Correlation coefficient (r)	
	trained panel	untrained panel
Canned "veronese"		
Colour – taste (in light)	0.823*	0 829*
Colour – taste (in the dark)	0.282	0.298
Taste (in light) – taste (in the dark)	0.243	0.399
Canned ham		
Colour – taste (in light)	0.284	0.577
Colour – taste (in the dark)	0.179	0.220
Taste (in light) – taste (in the dark)	0.010	0.161

*: Significant at P = 95% probability level (r = 0.754)

Table 7

First test results of Tristimulus values of tinted "pariser" slices
(Selected by the panels)

Sample	a*		b*		L*		C*		ΔE^*_{ab}
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	
Trained panel									
Natural colour	15.38	0.74	19.71	0.64	58.78	0.76	25.01	0.90	Basis of comparison
red: 0.01%	17.75	0.61	21.13	0.53	58.68	0.63	27.60	0.56	2.76
0.05%	22.23	0.75	23.99	0.88	56.41	0.74	32.71	1.09	8.42
green: 0.0025%	13.84	0.74	18.91	0.78	58.08	0.61	23.43	1.04	1.87
0.005%	11.43	0.70	18.80	0.42	56.41	0.57	22.00	0.66	4.70
rum brown: 0.03%	18.00	0.54	21.56	0.57	57.02	0.63	28.09	0.73	3.66
0.07%	19.04	0.53	22.47	0.76	55.63	0.71	29.46	0.78	5.56
Untrained panel									
Natural colour	13.81	0.57	16.83	0.54	63.25	0.69	21.78	0.44	Basis of comparison
red: 0.01%	16.42	1.12	18.22	0.53	61.43	0.49	24.55	0.63	3.47
0.05%	19.79	0.72	21.68	0.38	60.26	0.82	29.36	0.75	8.26
green: 0.0025%	11.37	1.09	16.25	0.75	62.92	0.50	19.87	0.48	2.53
0.005%	10.95	1.67	15.49	0.66	61.54	0.83	19.03	0.60	3.59
rum brown: 0.03%	16.58	1.30	18.95	0.34	60.28	0.88	25.21	0.71	4.58
0.07%	17.26	0.42	20.69	0.33	59.39	0.60	26.94	0.37	6.46

Table 8

Second test results of Tristimulus values of tinted "pariser" slices

Sample	a*		b*		L*		C*		ΔE^*_{ab}
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	
Trained panel									
Natural colour	16.87	1.68	13.45	0.84	54.91	1.04	21.60	1.66	Basis of comparison
red: 0.01%	16.66	0.98	15.56	0.81	54.93	0.78	22.82	0.73	2.12
0.05%	22.04	2.66	17.28	1.07	53.75	0.77	28.08	1.85	6.45
green: 0.0025%	10.91	1.17	11.41	0.77	53.34	1.14	15.81	1.09	6.49
0.005%	6.53	0.81	11.07	0.87	51.53	0.62	12.88	0.88	11.14
rum brown: 0.03%	15.24	1.82	13.67	0.52	55.60	0.68	20.50	1.57	1.78
0.07%	16.86	0.93	16.77	0.39	53.71	0.89	23.78	0.72	3.53
Untrained panel									
Natural colour	9.30	1.26	13.20	0.84	59.32	0.81	16.21	0.28	Basis of comparison
red: 0.01%	11.53	0.60	14.27	0.94	59.19	1.11	18.37	0.69	2.48
0.05%	14.39	6.77	18.55	2.75	57.35	1.77	24.42	1.44	7.64
green: 0.0025%	6.57	1.60	11.97	0.82	57.77	1.16	13.75	0.49	3.37
0.005%	3.76	1.24	12.15	0.47	57.47	0.53	12.76	0.74	5.93
rum brown: 0.03%	10.02	0.81	14.57	0.51	48.14	0.46	17.70	0.50	1.95
0.07%	12.67	1.39	18.07	3.72	56.87	0.54	22.16	3.34	6.41

Table 9

Values of correlation coefficient received by the linear regression analyses between the sensory colour and the objective colour values of tinted "pariser" slices

Sensory colour	Correlation coefficient (r)	
	trained panel	untrained panel
1st test		
a*	0.712	0.768*
b*	0.585	0.596
L*	0.250	0.556
C*	0.655	0.694
2nd test		
a*	0.727	0.757*
b*	0.650	0.451
L*	0.907**	0.519
C*	0.706	0.572

Number of panelists (n) = 7
*: Significant at P = 95% probability level (r = 0.754)
**: Highly significant at P = 99% probability level (r = 0.874)

The correlation coefficient of these tests are summarized in Table 6.

In the case of the mash made of canned "veronese" a close correlation was found between the colour and the taste, when judged in light by both the trained and the inexperienced panel. No correlation was found between colour and taste, when scored in darkness and between tastes when estimated in light and in darkness.

In the case of the mash made of canned ham and meat significant correlation was not found between the two characteristics when tested by either of the panels. However, the difference was substantial in the judgement of the inexperienced panel when the colour and the taste, tested in light and the colour and the taste, tested in darkness, were compared. In the former case the correlation coefficient was $r = 0.579$, while in the latter it was $r = 0.220$. This shows also the influence of colour on estimating the taste value.

2.2. Comparison of sensory and instrumental evaluation of colour

In further experiments the correlation between the sensory colour scores and the instrumentally determined colour sensation values, was studied. The parameters of the tinted "pariser" slices as determined in the CIELAB colour measurement system are shown in Tables 7 and 8. These tables show also the colour differences (ΔE^*_{ab}) calculated to be able to judge better the

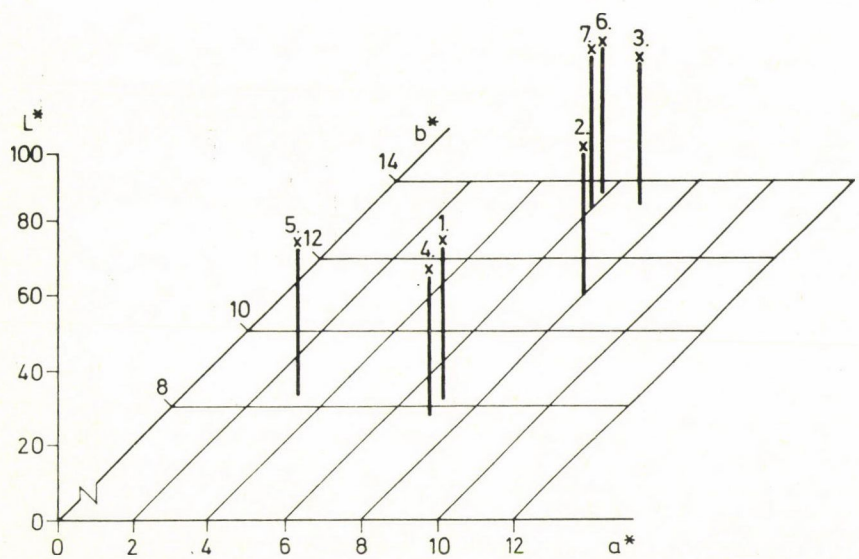


Fig. 2. Colour values of "pariser" slices in the CIELAB colour space. (Samples of the trained panel, 2nd test.) 1: Natural colour; 2: tinted with 0.01% red; 3: tinted with 0.06% red; 4: tinted with 0.0025% green; 5: tinted with 0.005% green; 6: tinted with 0.03% rum brown; 7: tinted with 0.07% rum brown

Table 10

Test results of Tristimulus values of canned ham samples

Sample	a*		b*		L*		C*		ΔE^*_{ab}
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	
Natural colour	7.00	0.64	8.22	0.56	42.89	4.01	11.28	0.37	Basis of comparison
red: 0.125%	7.97	2.91	10.97	0.49	41.13	1.48	13.71	1.88	2.94
0.25%	7.99	0.12	12.42	0.33	40.74	0.06	14.77	0.32	4.31
green: 0.01%	6.83	2.99	7.89	1.00	39.03	1.26	10.71	1.64	3.97
0.0375%	2.96	0.30	8.43	0.52	40.59	3.60	8.94	0.58	4.67
rum brown: 0.5%	6.71	0.36	12.77	0.16	41.04	0.78	14.43	0.29	4.23
1.0%	6.08	0.15	13.25	0.60	42.79	3.03	14.58	0.60	4.53

differences induced by tinting. Since the sensory tests could not be carried out for technical reasons by the trained panel and the inexperienced panel at the same time, the samples were prepared in exactly the same way, however, not from the same raw material. All the samples were submitted to colour measurement. The scores given the quality characteristics by the trained panel in the 2nd test were plotted also in the CIELAB colour space (Fig. 2).

As shown by the results the artificial intervention caused change in all the colour stimuli. The colour differences between the untinted and tinted samples was observable by the naked eye.

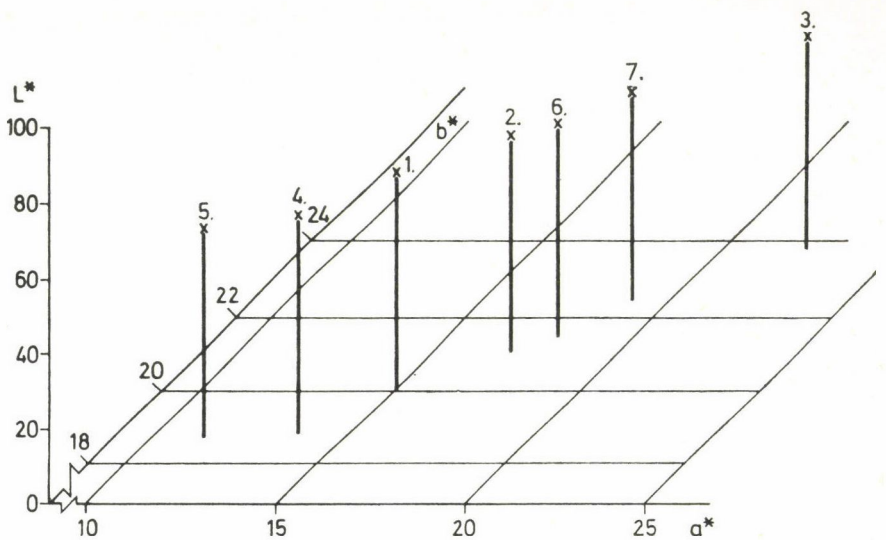


Fig. 3. Colour values of canned ham in the CIELAB colour space. 1: Natural colour; 2: tinted with 0.125% red; 3: tinted with 0.25% red; 4: tinted with 0.01% green; 5: tinted with 0.0375% green; 6: tinted with 0.5% rum brown; 7: tinted with 1.0% rum brown

Table 11

Values of correlation coefficients reveived by the linear regression analyses between sensory colour scores and the objective colour characters of canned ham samples

Sensory colour	Correlation coefficient (r)	
	trained panel	untrained panel
a*	0.852*	0.959**
b*	0.651	0.572
L*	0.277	0.328
C*	0.836*	0.785*

Number of panelists (n) = 7
*: Significant at P = 95% probability level (r = 0.754)
**: Highly significant at P = 99% probability level (r = 0.874)

The results of the analysis of rank correlation between sensory colour values and colour values in the CIELAB system are presented in Table 9.

A close and significant correlation was found between the sensory colour and the L* values. The correlation between sensory colour and value a* was only medium close.

Instrumental colour measurement and the correlation analyses between subjective and objective colour evaluation were carried out on the mashed meat samples, too. In the samples of canned "veronese" evaluable correlations were not found (probably due to error in measurement), therefore the related results are not given. The results of colour measurement in the canned ham samples are presented in Table 10 and Fig. 3 and the correlation coefficients obtained in Table 11.

The results are similar to those obtained in relation to "pariser" samples. In this case the correlation between sensory colour and the value of a* was found close and significant. A correlation with chroma was also observed.

3. Conclusions

Sensory evaluation and parallel instrumental colour measurement in samples of sliced fresh sausage called "pariser" and of mashed canned meat of uniform colour and tinted with tasteless food colouring agents permit of conclusions as follows.

In the case of samples tinted with unusual colour, influenced by the colour the tastes were scored by panel members differently, even if the taste was uniform. The latter was proven by tests carried out in the dark.

The colour of "pariser" samples tinted intensely green was scored by both panels significantly worse at the 99% probability level. The taste of the same samples was also scored lower at the 95% probability level on several

occasions. If testing was carried out in darkness such difference was not observed.

A similar tendency was found in the case of the mashed samples, however, the differences were not significant.

Samples ranked high in relation to colour were generally ranked high also in relation to taste, but differences were not significant.

The above findings seem to suggest that the greenish colour reminding of spoilage awoke the illusion of bad taste, too, with most of the panel members. At the same time a reddish or brownish tint, not differing from the natural, did not cause aversion.

The untrained panel members were always more affected by conditions. The results of these experiments are in accord with those of KOSTYLA and CLYDESDALE (1978) who found colour to affect quantitatively flavour and thus the colour is an extremely important factor in the judgement of foodstuffs. That is why the investigation of the psycho-physical relations of colour and taste is so very important.

The above findings are also supported by the correlation tests. In most of the cases a significant linear correlation was found between scores for colour and taste.

These observations agree with earlier experiences gained on studying foods of plant origin (URBÁNYI, 1982).

On the basis of this study the following can be concluded:

In the course of scoring the judgement of taste is influenced by the colour of the sample. Inexperienced panel members are more influenced than trained members. Thus, it is advisable to select sensory panels from experienced members or provide proper training for them.

Objective evaluation of taste can be promoted by measures encumbering the comparison of colours, e.g. scoring in darkness or in illumination covering colour differences or uniform tinting all the samples. Naturally in this case the colour must be tested separately.

In most of the sensory evaluations the values assigned to taste were in correlation with value "a*", characteristic of red.

The above observations suggest that it is advisable to carry out in parallel with the sensory evaluation instrumental colour measurement because their results may support the results of sensory tests and perhaps the threshold values of the colour characteristics determined on the basis of sensory evaluation can be utilized in classification.

Literature

AMERINE, M. A., PANGBORN, R. M. & ROESSLER, E. B. (1965): *Principles of sensory evaluation of food.* Academic Press, New York.

ANON. (1980): Food colors. *Fd Technol., 34,* 77–83.

BOOMANN, M. V. & EBERBACH, K. (1976): Einfluss der Lichtart auf Farbe und Bewertung von Fleischwaren. *Fleischwirtschaft, 56,* 510–513.

CHRISTENSEN, C. M. (1983): Effects of color on aroma, flavor and texture judgements of foods. *J. Fd Sci., 48,* 787–790.

ELBE, J. H. & JOHNSON, C. E. (1971): Sales pattern changing color may be a factor. *Canner/Packer, 140,* (8), 10. — Ref.: KOSTYLA, A. S. & CLYDESDALE, F. M. (1978).

HEATH, H. (1985): Sensory evaluation: an update. *Food,* (9), 28–33.

HOOD, D. E. T. (1986): A húsminőség érzékszervi sajátosságai. (Sensory characters of meat quality.) *Magy. Állatorv. Lap.,* (3), 175–179.

JOANES, D. N. (1985): On a rank sum test due to Kramer. *J. Fd Sci., 50,* 1442–1444.

KOSTYLA, A. S. & CLYDESDALE, F. M. (1978): The psychophysical relationships between color and flavor. *CRC Crit. Rev. Fd Sci. Nutr., 10,* 303–321.

KRAMER, A. (1963): Revised tables for determining significance of difference. *Fd Technol., 17,* 1596.

MAGA, J. A. (1973): Influence of freshness and color on potato chip sensory preferences. *J. Fd Sci.,38,* 1251–1252.

MAGA, J. A. (1974): Influence of color on taste thresholds. *Chem. Senses Flavor, 1,* 115–119.

MCLELLAN, M. R., KIME, R. W. & LIND, L. R. (1984): Relationships of objective measurements to sensory components of canned applesauce and apple slices. *J. Fd Sci., 49,* 756–758.

MOIR, H. C. (1936): Some observation on the appreciation of flavour in foodstuffs. *Chemy Ind., 55,* 145–148.

MONTEJANO, J. G., HAMANN, D. D. & LANIER, T. C. (1985): Comparison of two instrumental methods with sensory texture of protein gels. *J. Texture Stud., 16,* 403–424.

MOSKOWITZ, H. R., KAPSALIS, J. G., CARDELLO, A. V., FISHKEN, D., MALLER, O. & SEGARS, R. A. (1979): Determining relationships among objective, expert, and consumer measures of texture. *Fd Technol., 33,* (10), 84–88.

PANGBORN, R. M., BERG, H. W. & HANSEN, B. (1963): The influence of sweetness in dry table wine. *Am. J. Psychol., 76,* 492–495.

SAWYER, F. M., CARDELLO, A. V., PRELL, P. A., JOHNSON, E. A., SEGARS, R. A., MALLER, O. & KAPSALIS, J. (1984): Sensory and instrumental evaluation of snapper and rockfish species. *J. Fd Sci., 49,* 727–733.

URBÁNYI GY. (1982): Investigation into the interaction of different properties in the course of sensory evaluation. — Part I. The effect of colour upon the evaluation of taste in fruit and vegetable products. *Acta Alimentaria, 11,* 233–243.

Acta Alimentaria, Vol. 18 (2), pp. 143—151 (1989)

BIOCHEMICAL CHANGES IN STORED PEARS AFTER RADIATION, $CaCl_2$ AND KINETIN TREATMENTS

I. LIPOXYGENASE ACTIVITY IN RELATION TO THE BREAKDOWN DISORDER

S. Farag,[a] H. Daood and E. Kovács

Central Food Research Institute, H-1022 Budapest,
Herman Ottó út 15. Hungary

(Received: 4 December 1987; accepted: 18 August 1988)

Lipoxygenase (LOX) activity in pear fruits (var. Hardenpont) stored at 17 °C and 80–85% R. H. was determined after different treatments (irradiation with 1 and 2 kGy; $CaCl_2$ and kinetin). Activity levels were different in peel, flesh and core of the fruit and increased by the end of the storage period (90 days). Healthy parts of the fruits showed higher enzyme activity than those with internal breakdown. At the beginning of storage, enzyme activity was lower in irradiated fruits than in those without irradiation, whereas no considerable effect of the other treatments on LOX activity was observed. By the end of storage, $CaCl_2$ treatment caused strong activation of the LOX enzyme while the lowest activity level was noticed after kinetin treatment. The decay (%) of the fruits reached a high level, in agreement with the increase in LOX activity. $CaCl_2$ treatment promoted both LOX activity and decay (%) whereas irradiation caused substantial reduction in both. In spite of its remarkable inhibiting effect on LOX activity, kinetin treatment had no influence on decay (%) as compared to the untreated control.

Keywords: lipoxygenase, pear, gamma radiation, kinetin, calcium chloride

Lypoxygenase (linoleate: O_2 oxidoreductase E.C. 1.13.1.13) catalyzes the hydroperoxidation of poly-unsaturated fatty acids and esters containing a cis-cis-1,4-pentadiene system. The resulting 9-and 13-hydroperoxides are reported to exist in a ratio 3 : 7 in pear fruit (Kim & Grosch, 1979). The physiological disorder or the breakdown of apple fruits are complicated processes including high levels of LOX activity, especially in the core part (Feys et al., 1980). This has been associated with the role of LOX in the biogenesis of ethylene (Wooltorton et al., 1965).

Calcium chloride is widely used, as postharvest treatment, to reduce softening, respiration, ethylene evolution, development of bitter pit and breakdown of apple and pear fruits (Al-Ani, 1978 and Bangerth, 1979). In order to delay ripening of pear fruits gamma irradiation has been used in extended investigations by many workers (Salunkhe et al., 1959 and Maxie et al., 1971).

[a] Present address: National Centre for Radiation Research and Technology. Nasr City, P. O. Box 2. Cairo. Egypt

Akadémiai Kiadó, Budapest

The present work was undertaken to study the relationship between LOX activity and the physiological disorders resulting from different treatments with the ultimate goal of improving the quality of stored pear fruits.

1. Materials and methods

1.1. Materials

Pear fruits *(Pyrus communis* L. Var. Hardenpont) were obtained from a Farmer's Cooperative, Nagykanizsa (Hungary). Linoleic acid and Tween-20 were from Sigma (USA). The other chemicals used were from Reanal (Hungary).

1.2. Fruit treatment

The ripe fruits (at technological ripeness) were taken and divided into four groups. The first was treated by gamma radiation at a dose rate of 1 and 2 kGy per h. The second was dipped for 10 min in a 2% solution of $CaCl_2$. The third group was dipped for 10 min in a 20 mg dm^{-3} kinetin solution. The untreated (control) group was the fourth one. The fruits were stored at 17 °C and 80–85% R. H. in open plastic cases (the traditional conditions of fruit storage in Hungary). Random samples were taken at the beginning and at the end of storage for measurement of LOX activity. For studying the effect of treatments on pear firmness, the fruits were stored at 4 °C and randomly selected samples were taken every 3 months for measurement. It is to be mentioned that the treatments were repeated twice and 2–4 replicates were done in each analysis.

1.3. Determination of LOX activity

1.3.1. Crude enzyme preparation. The enzyme was extracted from the different parts of the fruit according to the method of Kim and Grosch (1979) with some modifications. Fifty gram samples of peel, flesh and core parts were disintegrated in a mortar with quartz sand. The disintegrated samples were then stirred with 0.1 mol l^{-1} TRIS-HCl buffer (pH = 6.2) containing 0.2% Triton X-100 and 0.1% $Na_2S_2O_5$. The mixture was strained through double layer cheese cloth and the initial homogenate obtained was centrifuged for 20 min at 15 000 r.p.m. (Beckman type JA-20, USA). The clear supernatant was used as the crude extract of LOX.

1.3.2. Substrate preparation. Linoleic acid substrate was prepared according to the method of Sekhar and Reddy (1982) which was then further developed by Daood and Biacs (1986). The substrate should be flushed with O_2 for 2 min before mixing with the reaction mixture.

1.3.3. Assay of the enzyme activity. A spectrophotometric method based on determining the increase in absorbance at 234 nm was used to assay LOX activity according to AL-OBAIDY and SIDDIQI (1981). The method involved initiation of the reaction in a spectrophotometric cell by adding 50 μl of the enzyme extract to the reaction mixture containing 5.0 μl of substrate and 2.9 cm^3 of 0.1 mol l^{-1} phosphate buffer (pH = 6). The blank contained heat-inactivated enzyme extract. One unit of enzyme is defined as that amount which produces a change in the value of 0.01 absorbance per min at 234 nm under the assay conditions.

1.4. Firmness and decay

The softening of fruits was measured by a penetrometer (DP-O2 type, Dispersitron, Hungary). The firmness was evaluated for whole fruit with and without skin; 16 fruits were used in every treatment. The deteriorated fruits (with internal breakdown) were removed monthly, and calculated as the percentage of decay. The values given as results present means of 3–4 replicates.

2. Results and discussion

2.1. Occurrence of LOX in pear fruit

It is a well-known fact that the different parts of the fruit contain different levels of enzyme activity. The richest one, of course, would likely be of special interest when the study aims at investigating the effect of different treatments of the enzyme activity and on its manner during storage and processing as well. In this work the analyses showed that flesh part of freshly harvested fruit show most of LOX activity (Table 1). Therefore, LOX activ-

Table 1

LOX activity in different parts of pear fruit during storage

Part of fruit	LOX activity unit (g fresh weight)			
	after 15 days		after 90 days	
	$\bar{x}$	±s	$\bar{x}$	±s
Peel				
healthy	75	3.7	475	23.6
black spotted	27	0.81	80	2.8
Flesh	218	10.9	275	13.8
Core	148	6.7	350	9.6

The values were obtained from the untreated fruits

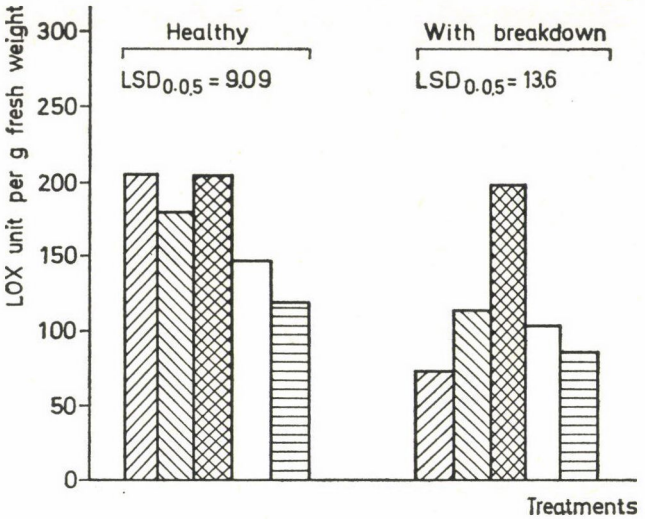


Fig. 1. LOX activity in flesh of pear fruits after 15 days of storage at 17 °C, 80–85% R. H. ▨: control, ▨: $CaCl_2$, ▨: kinetin, ▭: 1 kGy, ▨: 2 kGy. LSD: least significant difference

ities in flesh of the treated and the control samples were compared. It was also found that at the end of the storage period LOX activity increased in all parts of fruit but was the highest in the peel. Lower LOX values were measured in the black spots than in the normal or healthy peel. This is probably due to the LOX inhibitory effect of phenolic compounds accumulated at the dark spots and exist at considerable amount in the extract of unhealthy peel of pear fruits. Similar observation has been reported on the occurrence of LOX in the different parts of apple and its activity at preclimacteric and climacteric of the fruits (FEYS et al., 1980).

2.2. *Effect of postharvest treatment on LOX activity and its relation to the quality of fruits*

The use of gamma rays (2 kGy) decreased LOX activity as shown in Figs. 1 and 2. The LOX activity was lower in fruits irradiated with 2 kGy than in 1 kGy samples both with healthy and black spotted flesh. These results indicate the inhibition of ripening by gamma rays, reported to be due to the enzyme system in mitochondria or to ethylene production in pear fruits (Fig. 3). Linolenic acid is one of the intermediates in the biogenesis of ethylene production (ESKIN et al., 1977). Gamma irradiation decreased the blackening phenomenon. Two KGy also decreased the internal breakdown or decay (%). Beside that, irradiated pears (with or without skin) held the

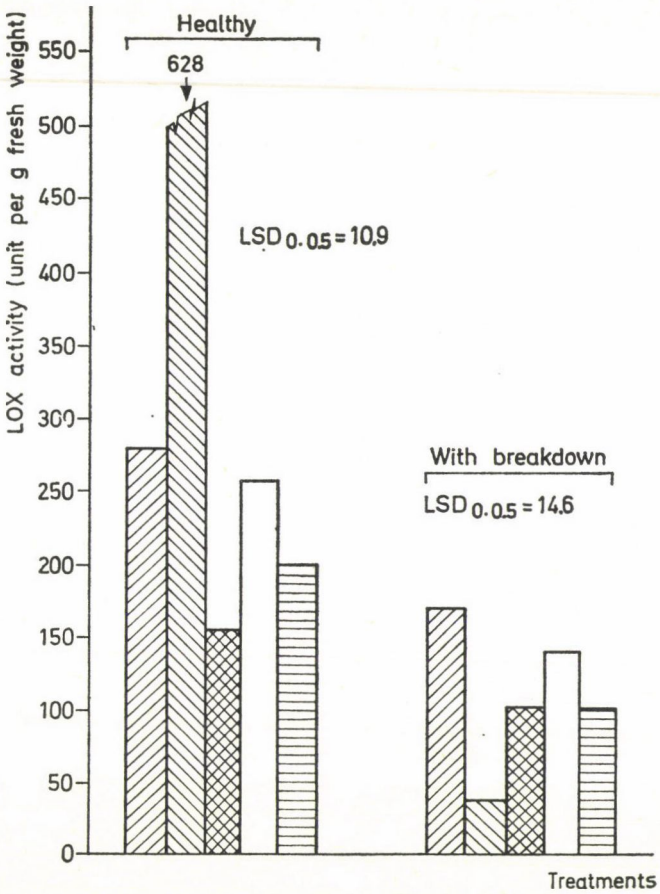


Fig. 2. LOX activity in flesh of pear fruits after 90 days of storage at 17 °C, 80–85% R. H. ▨: control, ▨: $CaCl_2$, ▨: kinetin, ▭: 1 kGy, ▨: 2 kGy. LSD: least significant difference

second rank in the development of softening (Figs. 4 and 5). All these results confirm the role of gamma irradiation in delaying the senescence of pear fruits as reported by SALUNKHE and co-workers (1959), and MAXIE and co-workers (1971). The control sample decayed faster than the irradiated one. More than 50% of the 2 kGy treated sample was healthy after 90 days of storage, compared to 35% of the control

The penetration of $CaCl_2$ through the flesh parts caused strong activation of LOX, and an increased decay to 80% by the end of storage, while the remaining part (20%), i.e. the healthy fruits, were firmer than the former with other treatments.

It was observed that the appearance of black spot was more rapid on the fruit in which high activation of LOX occurred than on those of low LOX

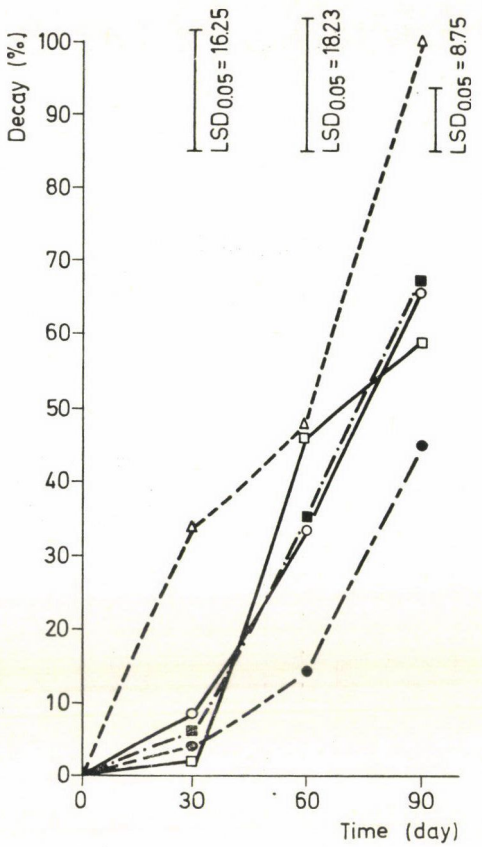


Fig. 3. Effect of different treatments on decay (%) of stored pear fruits. ○—○ : control, △---△ : $CaCl_2$, □—□ : kinetin, ■—■ : 1 kGy, ●—● : 2 kGy. LSD: least significant difference

activity. This observation may help to explain why some cultivars of pear, i.e. Hardenpont, are very sensitive to skin blackening. This phenomenon starts usually before harvest and gradually increases during handling and storage at high temperature or upon the fluctuation of storage conditions. The black spots may change to internal breakdown in the flesh part (GYURÓ, 1976).

This confirms the firmness increasing role of $CaCl_2$ in fruit and vegetables. However, this treatment can cause high activation of LOX which promotes the internal breakdown (ABBAS et al., 1979; and ESKIN et al., 1977). Increased O_2 consumption induced by $CaCl_2$ in apple flesh tissues has been observed by HIMELRICK and INGLÆ (1981). However, they could not explain it. The O_2 consumption and increased respiration could be explained by the change in linoleic acid which produces hydroperoxides with LOX. This reaction can be promoted by $CaCl_2$ (ESKIN et al., 1977).

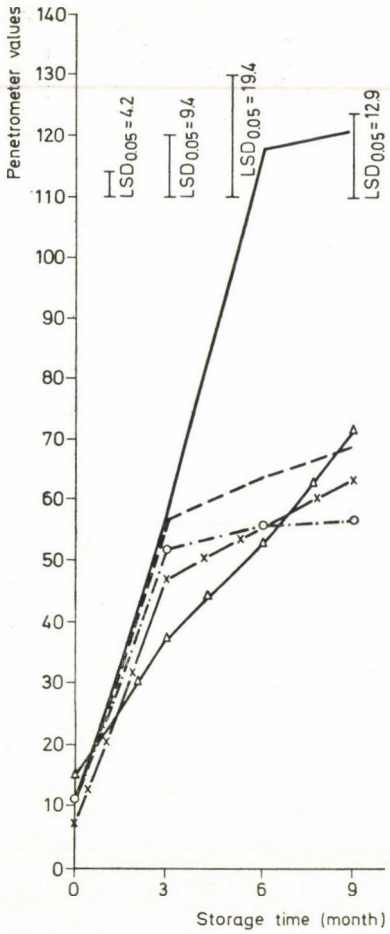


Fig. 4. Softening of pear fruits (without skin) during cold storage (4 °C, 80–85% R. H.) ———: control, - - - - -: $CaCl_2$, - - - - -: kinetin, —×—: 1 kGy, —▲—: 2 kGy. LSD: least significant difference

LOX activities in deteriorated fruits did not confirm those observed in healthy samples. The difference between treatments at the beginning as well as at the end of storage were not understood and no explanation could be made.

3. Conclusion

It could be concluded that LOX activity is related to the physiological breakdown disorders. LOX activity can be inhibited by gamma rays or by kinetin treatment and the storeability of the fruits can accordingly be

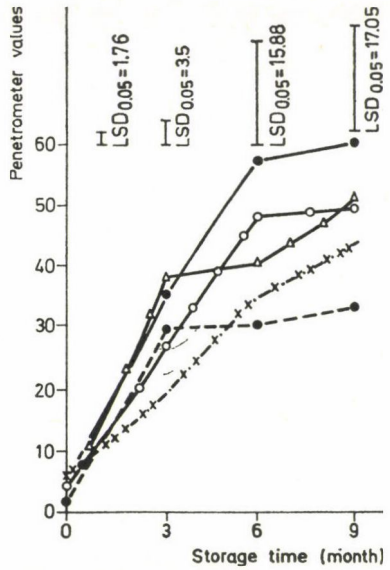


Fig. 5. Softening of pear fruits (with skin) during cold storage (4 °C, 80–85% R. H.) ———: control, - - - - -: $CaCl_2$, —o—: kinetin, —▲—: 1 kGy, —×—: 2 kGy. LSD: least significant difference

increased. $CaCl_2$ treatment which is known to increase the firmness of fruits failed to stop the internal breakdown, which is a complicated process involving LOX activity.

Literature

ABBAS, I. R., SIDDIQUI, A. M. & TOAMA, S. J. (1979): Broad been lipoxygenase. — Part I. Partial purification and characterisation. *Fd Chem.*, *4*, 269–281.

AL-ANI, A. M. (1978): Postharvest physiology of "Anjou" pear fruit: Relation between mineral nutrition and cork spot, respiration and ethylene evolution. Ph. D. Thesis, Oregon State Univ. Corvallis, Oregon, USA.

AL-OBAIDY, H. M. & SIDDIQUI, A. M. (1981): Inhibition of broad bean lipoxygenase. *J. Fd Sci.*, *46*, 597–605.

BANGERTH, F. (1979): Calcium-related physiological disorders of plants. *Rev. Phytopathol.*, *17*, 79–122.

DAOOD, H. & BIACS P. A. (1986): Evidence for the presence of lipoxygenase and hydroperoxide-decomposing enzyme in red pepper seeds. *Acta Alimentaria*, *15*, 307–318.

ESKIN, N. A., GROSSMAN, S. & PINSKY, A. (1977): Biochemistry of lipoxygenase in relation to food quality. *CRC Crit. Rev. Fd Sci. Nutr.*, *9*, 1–40.

FEYS, M., NAESENS, W., TOBBACK, E. & MAES, E. (1980): Lipoxygenase activity in apples in relation to storage and physiological disorders. *Phytochem.*, *19*, 1009–1011.

GYURÓ, F. (1976): *Körte.* (Pear.) Mezőgazdasági Kiadó, Budapest, pp. 1–395.

HIMELRICK, D. G. & INGLE, M. (1981): Effects of calcium, EDTA and oxalic acid on respiration of apple slices. *HortScience*, *16*, 165–167.

KIM, I. & GROSCH, W. (1979): Partial purification of a lipoxygenase from apples. *J. agric. Fd Chem*, *27*, 243–246.

MAXIE, E. C., SOMMER, N. F. & MITCHELL, F. G. (1971): Infeasibility of irradiating fresh fruits and vegetables. *HortScience*, *6*, 202–204.

SALUNKHE, D. K., GERBER, P. K. & POLLARD, L. H. (1959): Physiological and chemical effects of gamma radiation on certain fruits and vegetables and their products. *Proc. Am. Soc. hort. Sci., 74,* 423–429.

SEKHAR, B. P. S. & REDDY, G. M. (1982): Studies on lipoxygenase from rice. *J. Sci. Fd Agric., 33,* 1160–1163.

WOOLTORTON, I. S. C., JONES, J. D. & HULME, A. C. (1965): Genesis of ethylene in apples. *Nature, 207,* 999–1005.

Acta Alimentaria, Vol. 18 (2), pp. 153–166 (1989)

THE CHEMICAL COMPOSITION OF PRESSED AND EXTRACTED APPLE JUICE

GY. PÁTKAI and SZ. TÖRÖK

Department of Canning Technology, University of Horticulture and Food Industry, H-1118 Budapest, Ménesi út 45. Hungary

(Received: 21 December 1987; accepted: 18 August 1988)

On the basis of the experiments carried out on the quality of the juices of three different apple sorts (Jonathan, Golden Delicious and Starking) it can be established that the composition of the product gained by extraction at 56–58 °C differs from the pressed product in the following:

— the saccharose content is lower;

— the reducing sugar content relative to dry matter is higher,

— on the whole, the total sugar content in dry matter is the same as in the pressed juice;

— the acid content of the extracted juice is lower by about 5–10 relative percent;

— due to the inactivation of the enzymes the pectin and polyphenol contents are higher than in the pressed juice;

— the colour and moist fibre content of the juice extracted from Jonathan apples is of better quality;

— if condensed water is used for extraction the ash content does not differ from that of the pressed juice. If extraction is carried out with tap water the ash content of the extracted juice is higher than that of the pressed juice.

The above findings vary with the different apple varieties as follows:

— in Jonathan juice only the favourable characteristics — light colour, low fibre content — were proven by mathematical statistical calculations. This observation was supported also by sensory tests;

— the increase in pectin content is higher in Starking juice than in that of the Jonathan or Golden Delicious varieties;

— reduction of acid content was highest in the juice of Golden Delicious. The extracted juice of this variety was darker than the pressed juice.

Increasing the extraction temperature from 50 to 60 °C affected the general conclusions in the following ways:

— the advantages of the extracted juice (higher sugar content relative to dry matter, lighter colour) over the pressed juice diminished while setbacks (darkening of colour, reduction of acid content) were caused.

Finally, with sound selection of variety — Jonathan seems to be the most suitable —, by choosing the most appropriate extraction temperature (56–58 °C) and suitable clarifying technology it is possible to obtain an extracted apple juice and a concentrate of good quality (PÁTKAI, 1986).

Keywords: apple juice, diffusion, extraction

Apple juice manufacture based on the principle of diffusion extraction has awoken the interest of the experts already at an early stage of development. The advantages observed were: an increase of 15–20% in the dry matter yield over the pressed juice; continuous operation and good adaptability to automation (OTT et al., 1960).

Akadémiai Kiadó, Budapest

Although these advantages are achieved only at the cost of higher energy consumption and higher investment, it has been shown that on the whole juice production directly from apple slices by extraction has definit economic advantages over the traditional pressing method.

A number of unresolved problems hinder the spreading on a large scale of this technique, among others the quality of the juice thus obtained is queried.

In FRG it is ruled that only juices obtained by a mechanical process (e.g. pressing) can be considered as fruit juice. The same applies to concentrates made of juice and to imported fruit juices, too.

The guiding principles of EEC (European Economic Community) leave it to the member states to grant permission. Some of the member states e.g. Denmark permits juice production by extraction for the purpose of concentration (ANON., 1977).

At the joint meeting in 1984 of Codex Alimentarius Committee and United Nation/European Economic Council a new proposal was submitted to the governments of the member states in which it was proposed to consider exclusively the quality of the juice as basis for permission and not the production technology (TRENKLE, 1984).

The aim of the present work is to summarize the available literary data and current experiences gained on the quality of apple juice manufactured by direct extraction. Research results published on this subject are surveyed in the following.

The first and most important requirement of introducing direct extraction is that the quality of the juice and concentrate thus produced should reach or even exceed that of the traditionally pressed juice.

According to several papers a lower percentage of the dry matter content of the juice produced by extraction is provided by sugar than in the pressed juice (WUCHERPFENNIG et al., 1976; SCHOBINGER et al., 1978).

CONCEN and co-workers (1977) found that the reducing sugar content of the extracted juice is somewhat higher, the glucose to fructose quotient slightly lower than in pressed juice.

WUCHERPFENNIG and POSSMANN (1979); WUCHERPFENNIG and co-workers (1976) and LÜTHI and GLUNK (1974) report that the titratable acid content of juices produced on large scale is lower than in pressed juices, while GOLDBACH and co-workers (1978) made just the opposite observation. Data obtained by DOUSSE and co-workers (1977) prove the acid content to be the same in juices produced by either of the two methods.

Several authors draw the conclusion that the pectin content of juices manufactured by extraction is higher than that of pressed juices (SCHOBINGER et al., 1978; LÜTHI & GLUNK, 1974; DOUSSE et al., 1977; CONCEN et al., 1977). On the other hand, it is the unanimous opinion of a number of authors that

the polyphenol content and the phloridin content, originating from the seeds is higher in the extracted juices than in the pressed juices (PEDEN, 1976; DOUSSE et al., 1977; WUCHERPFENNIG et al., 1979; LÜTHI & GLUNK, 1974; SCHOBINGER et al., 1978; GIERSCHNER et al., 1978; HAUG & GIERSCHNER, 1979).

Many data in the literature show that extracted juices are extremely rich in aromatic substances and aroma extracts gained from them are of high quality (LÜTHI & GLUNK, 1974; GHERARDI, 1978; LÜTHI & GLUNK, 1979). They particularly stress extremely high aroma content of the extract obtained from pomace by a cold process (SCHOBINGER et al., 1978). In sensory evaluations high polyphenol concentration can be observed in extracted juices. This slightly harsh character is, however, found by some panel members even refreshing.

As regards taste several authors (DOUSSE et al., 1977; SCHOBINGER et al., 1978; BINKLEY & WILEY, 1978) found the pressed juice better than the extracted juice. These authors carried out their experiments on laboratory size extractor. One paper mentions that the juice prepared on an Amos extractor had a very agreeable, fresh taste (ANON., 1977). KARDOS (1962) and LAURSEN (1974) extracted the juice of Starking apples on the pilot plant size Type DdS extractor and the sensory properties of this juice did not differ from that of the pressed juice.

It is interesting to compare the colour of extracted and pressed juices. DOUSSE and co-workers (1977), BINKLEY & WILEY (1978) and CONCEN and co-workers (1977) found the extracted juices lighter in colour than the pressed juices. On the other hand, SCHOBINGER and co-workers (1978) found the absorbance at 420 nm of the juice, prepared on laboratory apparatus higher than that of the pressed juice.

Several publications report the extracted juices to have a higher protein content than the pressed juices (SCHOBINGER et al., 1978; DOUSSE, 1977; GOLDBACH et al., 1978). Most of the related papers state that juices extracted at 60 °C are of higher microbiological stability and of a lower alcohol content than pressed juices (LÜTHI & GLUNK, 1974; 1979). These results seem to be contradicted only by those obtained by SCHOBINGER and co-workers (1978).

Measurements carried out by DOUSSE (1977) and WUCHERPFENNIG and co-workers (1976) show that the suspended material and fibre content are lower in the extracted juice than in the pressed juice.

As it can be seen from the above survey of related literature many problems were left unresolved.

1. Materials and methods

Jonathan, Starking and Golden Delicious edible apple varieties, obtained from the Model Farm, University of Horticulture and Food Industry, were used in the experiments.

1.1. Production of pressed juice

Apple mash was prepared by cutting up the apples manually, then using a Keripar EK-1000 type (Hungary) institutional kitchen size robot fitted with a meat mincer (of 0.4 cm perforation diameter). The 6–8 kg mash was pressed out on a laboratory size basket type winepress. To determine the juice yield the amount of mash, the pressed juice and the water soluble dry matter content were measured. The pressed juice was then quick-frozen and stored for later chemical analysis and sensory test.

1.2. Juice production by extraction

In order to create the active surface required to material transfer the apples were sliced either with the slicer fitting belonging to the EK-1000 kitchen robot or with the special knives of "waved" profile manufactured by the Urschel firm for apple slicing and fitted in the knife disc of the EK-1000 machine.

The equipment used for juice extraction was a continuously operated laboratory size single spiral extractor.

This equipment was manufactured by KÖVAC (Hungary) based on the technical data of the laboratory size machine of the Danish DdS firm, recommended for experimental purposes.

1.3. Methods of juice analysis

1.3.1. Water soluble dry matter content, titratable acid content, pH value, ash content, total and reducing sugar were determined according to the methods specified by pertinent HUNGARIAN STANDARDS (1971; 1974a; 1974b; 1983).

1.3.2. Glucose content. It was determined by an enzyme method with a set glucose-selective electrodes (Type OP-Gl-7113). The saccharose content was calculated from the difference between total sugar and reducing sugar content. To calculate the fructose content the difference between reducing sugar and glucose content was used.

1.3.3. Pectin content. It was determined in the form of Ca-pectate. The precipitate formed with $CaCl_2$ was filtered, dried to constant weight and weighed.

1.3.4. Colour of the juices and concentrates. A 5-time dilution of the centrifuged sparkling juice was used to measure the absorbance at 425 nm on the Zeiss Specol (GDR) spectrophotometer.

1.3.5. Raw protein content. The samples were macerated with sulfuric acid and a colour reaction was brought about with Na-Salicylate on the Labor MIM (Hungary) Contiflo instrument. By this method was determined the nitrogen content and by multiplying with factor 6.25 the protein content was calculated.

1.3.6. Total polyphenol content. To determine the polyphenol content the Folin-Ciccalteau reagent was used. The colour reaction was registered by photometry and a calibration diagram according to SINGLETON and ROSSI (1970) was plotted in the 0.0–2.5 mg cm^{-3} gallic acid concentration region. Thus, the result is obtained related to gallic acid.

1. 3.7. Moist fibre content. The samples were centrifuged for 20 min at 700 r.p.m. The sediment was washed with 50% ethyl alcohol. After repeated centrifuging the sediment was weighed.

1.3.8. Organoleptic evaluation. To be able to differenciate between pressed and extracted juices the triangular test was applied.

2. Results and conclusions

2.1. Comparison of juices gained by pressing and diffusion extraction

The average composition of the juices obtained by extraction and pressing from samples collected during two seasons is given in Table 1. The data are based on the analysis of a total of 19 Golden Delicious, 14 Jonathan and 12 Starking apple samples. (Average juice yield by pressing: 55%; average dry matter yield by extraction: $65 \pm 1.2\%$).

The following conclusions can be drawn from the comparison of extracted and pressed juices.

As regards total sugar content significant difference was not found between extracted and pressed juices either according to apple variety or in the summarized values.

The dry matter content free from sugar was not given as the difference between total dry matter and total sugar content. This would not have conveyed new information. The sugar-free dry matter content is given as the sum of titratable acid, ash and pectin contents obtained by measurement. (Since the polyphenol wet fibre and protein content was determined only in the samples of the 1981 season, they were not included in the calculated non-sugar dry matter content.)

As it can be seen in Table 1 the sum of titratable acid, pectin and ash content is significantly lower in pressed juices than in the extracted juices.

Table 1

Comparison of the composition of pressed and extracted apple juice

Parameters	Jonathan			Golden Delicious			Starking			Average		
	P	E	$SD_{5\%}$	P	E	$SD_{5\%}$	P	E	$SD_{5\%}$	P	E	$SD_{5\%}$
Extract (g per 100 g)	13.15	9.48	—	13.0	10.27	—	13.75	10.16	—	13.5	9.97	—
Total sugar (g per 100 g dry matter)	78.6	80.48	4.48	51.52	82.2	2.88	79.7	78.91	4.2	80.1	80.76	2 02
Sugar-free dry matter (g per 100 g dry matter)	9.33	9.87	1.11	6.67	8.20	0.73**	7.39	10.23	1.09**	7.64	9.17	0.45**
Reducing sugar (g per 100 g dry matter)	64.39	66.47	3.5	58.14	61.09	2.57**	61.29	64.16	2.9*	60.99	63.63	1.59***
Glucose (g per 100 g dry matter)	9.35	10.15	1.81	6.14	7.16	1.16*	8.47	9.49	1.78	7.73	8.69	0.79**
Fructose (g per 100 g dry matter)	55.03	56.26	2.94	52.0	53.88	2.77	52.82	55.30	3.2	53.20	55.03	1.55**
Saccharose (g per 100 g dry matter)	14.60	15.2	2.48	23.37	20.97	2.34**	18.41	14.73	3.89*	19.21	17.47	1.57**
Titratable acid (g per 100 g dry matter)	3.78	3.6	0.27	2.36	2.04	0.12***	1.65	1.52	0.11**	2.61	2.39	0.1***
Ash (g per 100 g dry matter) (1979) (1981)	1.87	1.88	0.32	1.77	1.84	0.3	1.39	1.55	0.18*	1.70 1.54	1.77 2.01	0.1 0.28***
Pectin (Ca-pectate) (g per 100 g dry matter)	3.34	3.99	0.86	2.77	4.29	0.8***	4.35	7.30	1.13***	3.41	5.00	0.48***
Polyphenols (g per 100 g dry matter)	0.114	0.61	0.1***	0.126	0.55	0.11***	0.14	0.31	—	0.12	0.56	0.08***
Protein (g per 100 g dry matter)	0.54	0.48	0.4	0.37	0.42	0.14	—	—	—	0.46	0.45	0.19
Adsorbance (1% at 425 nm)	0.15	0.076	0.042***	0.08	0.12	0.034**	0.166	0.142	0.032	0.123	0.113	0.023
Wet fibre (g per 100 cm^3)	0.274	0.0175	0.24**	0.65	0.231	0.81	—	—	—	0.5	0.15	0.46
pH	3.83	3.93	0.17	3.98	4.26	0.42	4.24	4.45	—	3.93	4.13	0.09**

P = pressed juice
E = extracted juice
P–E = difference between P and E

$SD_{5\%} = t_{5\%} \cdot s_{\bar{d}}$
$s_{\bar{d}}$ = standard deviation of the average difference between P and E

*: P–E significant at P = 10% probability
**: P–E significant at P = 5% probability level
***: P–E significant at P = 1% probability level

This applies to Golden Delicious and Starking varieties separately, too, at $P = 5\%$ probability level. Since there is no significant difference in the total sugar contents thus it must be true that pressed juices contain more non-detectable unknown colloidal substances and watersoluble compounds than extracted juices. This is supported also by the fact that the sum of the measured components approximates closer the actual dry matter content in the case of extracted juices than in pressed juices. The reducing sugar content of extracted juices related to their water-soluble dry matter content is significantly higher at $P = 1\%$ probability level than that of pressed juices. This is true for Golden Delicious and Starking varieties individually, too.

In conformity with the above the glucose and fructose content of the extracted juice is also higher at $P = 5\%$ probability level than that of the pressed juices. However, studying the individual varieties separately this is true only for Golden Delicious and only in relation to glucose.

The saccharose content in the extracted juices is significantly lower at $P = 5\%$ probability level than in pressed juices. This is true also separately for varieties Golden Delicious and Starking.

This can be interpreted as the hydrolysis of saccharose during extraction.

The titratable acid content of the juices decreased significantly as an effect of conditions of extraction. The titratable acid content of extracted juices is lower at $P = 1\%$ probability level than that of the pressed juices. On studying individual varieties the difference for Golden Delicious is at $P = 1\%$ probability level, and for Starking at $P = 5\%$ probability level, significant.

The ash content of each experimental series was separately evaluated because in the first series condensed water was used for extraction upon the recommendation of certain authors. In the second and third series potable tap water was used for extraction. The ash content of the juices can be highly affected by the quality of water used for extraction. On the whole, if condensed water was used no significant change was found between extracted and pressed juiced.

Using tap water for extraction the ash content of the extracted juice was at $P = 5\%$ probability level significantly higher than the ash content of the pressed juice.

As compared the colour of extracted juices with those of pressed juices interesting results were obtained, the varieties individually showed different character. In case of a mixture, made of approximately identical amounts of the three apple varieties used in the experiments, the difference observed in the colour of the pressed and extracted juices is non-significant.

The pectin content of the extracted juice was significantly higher at $P = 1\%$ probability level than that of the pressed juice. In individual assess-

ment of the varieties this finding proved to be valid only for Golden Delicious and Starking.

The pH value in the extracted juices is higher at $P = 1\%$ probability level than that in the pressed juice. This is in accordance with the lower acid content of the extracted juices.

The polyphenol, wet fibre and protein content as well as the pH value was determined only in one of the seasons. Thus, there were fewer samples available for comparison. In spite of this it was proven that the polyphenol content of the pressed juice was significantly lower at $P = 1\%$ probability level than that of the extracted juice. The difference when evaluated for each variety separately was significant, too.

As regards the wet fibre content a tendency towards a lower value was observed in extracted juices, this could be proven, however, only for the Jonathan variety, by mathematical statistical calculation. Since all the apple varieties were extracted in screw-drive extractor and the fruit itself was softened over a long storage period, it seems probable that from a better quality raw material an extractor conveying the slices more gently would have yielded a juice of lower suspended material content than in the pressed juice of the three apple varieties.

2.2. Composition of the extracted juices as affected by temperature

The average differences in the composition between the pressed and extracted juices as described above, relate to a temperature of 57 °C because this was the temperature generally applied in the experiments.

It was found necessary to investigate the chemical parameters also in relation to different extraction temperatures. This correlation was studied in a separate experimental series. In these experiments beside temperature only the shape of the slices (wavy surface or plain discs) varied, however, this did not affect the composition of the extracted juice.

Each of the three varieties was tested in 11 extraction experiments in the temperature range of 50 to 60 °C raising the temperature by 1–1.5 degrees.

The difference between the composition of the pressed and extracted juice was evaluated as a function of extraction temperature.

Deviation from the pertinent data of the control sample and its relation to extraction temperature was analysed by regression analysis only by fitting to the linear function.

In the knowledge of the correlation between extraction temperature and deviation from the control the correlation between the chemical parameters studied and the extraction temperature can be calculated, too.

In the case of data where regression analysis showed linear relation the

Table 2

Concentration of the chemical characters (y), extraction temperature (x) and equations describing their correlation
($n = 8$; $n = 11$; $n_G = 12$)

Chemical character (g per 100 g dry matter)	Variety	$y = mx + b$	Correlation coefficient (r) calculated from the table	
Total sugar	Jonathan	$y = -1.57x + 165.50$	−0.63	0.62 (P = 10%)
	Starking	$y = -1.42x + 159.81$	−0.72	0.68 (P = 2%)
Saccharose	Jonathan	—	—	—
	Starking	$y = -0.945x + 69.48$	−0.66	0.60 (P = 5%)
Fructose	Jonathan	—	—	—
	Starking	$y = -0.88x + 103.82$	−0.77	0.73 (P = 1%)
Glucose	Jonathan	—	—	—
	Starking	$y = 0.47x - 17.06$	0.64	0.60 (P = 5%)
Pectin	Jonathan	$y = 0.27x + 18.20$	−0.82	0.79 (P = 2%)
	Starking	$y = 0.20x + 17.55$	−0.63	0.60 (P = 5%)
Colour	Jonathan	$y = 0.01x + 0.48$	0.95	0.92 (P = 0.1%)
	Golden Delicious	$y = 0.015x - 0.67$	0.69	0.66 (P = 2%)

equations describing the correlation and the correlation coefficients are given in Table 2.

As it can be seen in the table, in relation to the dry matter total sugar, saccharose, fructose and pectin contents decreased with increasing extraction temperature while the glucose content and the colour showed an increasing tendency.

The correlation coefficients prove a loose correlation, only. This is because as an effect of the increasing temperature simultaneously several processes occur: Change in the rate of diffusion, transformation of various compounds, alterations in enzyme activity, etc. The results as measured show the resultant of all these processes. Figures 1 and 2 represent graphically these relationships, given in Table 2.

The most important conclusion drawn from the two diagrams that the composition of the juice extracted at 56–58 °C approximates most closely that of the pressed juice. The effect of temperature was not reflected in the juice of Golden Delicious variety therefore this was not illustrated.

The reduction in the ratio of the amount of sugar in the total water soluble dry matter content caused by increasing temperature may be the result of two different processes:

— reduction in the absolute value of sugar due to decomposition,

— relative reduction of sugar concentration in the total dry matter content, since at higher temperature increasing amounts of non-sugar compounds (pectin, proteins, colloids and decomposition products) become dissolved in the juice.

Analysis carried out as a function of extraction temperature, based

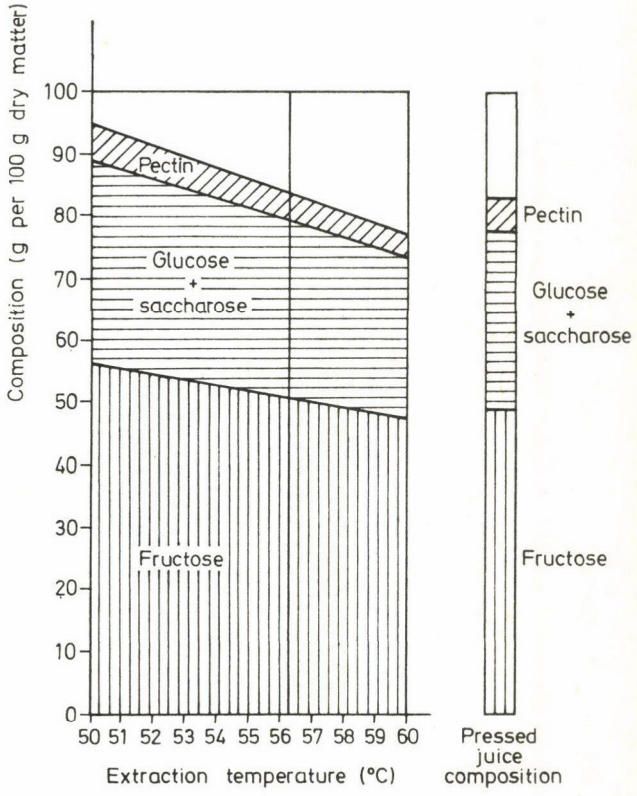


Fig. 1. Composition of apple juice extracted at temperatures between 50 to 60 °C (Variety Jonathan)

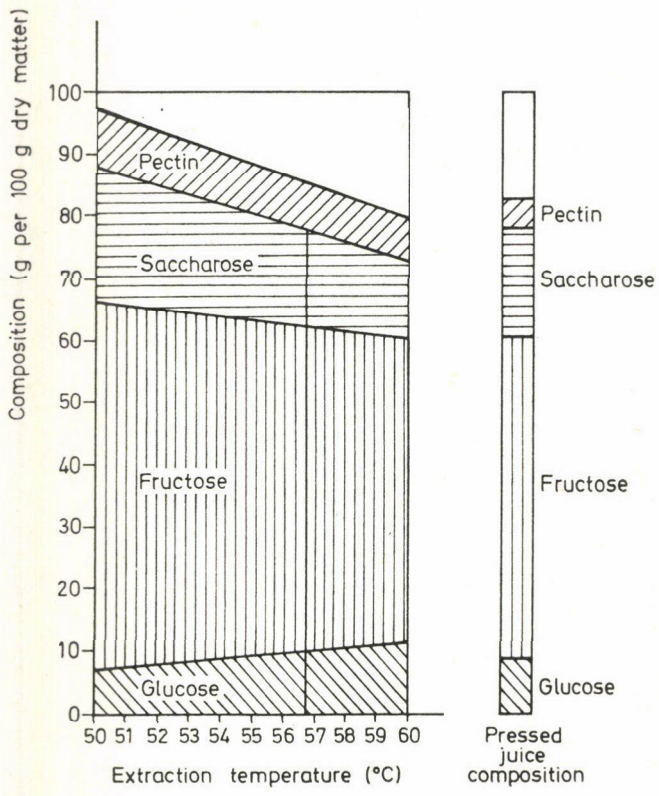


Fig. 2. Composition of apple juice extracted at temperatures between 50 to 60 °C (Variety Starking)

on the summarized average (related to 57 °C extraction temperature), shows that there is no significant difference in the total sugar content of the extracted and pressed juice. If, however, extraction is carried out at the lower temperature of 50–52 °C than the total sugar content of the extracted juice, related to its dry matter content is higher than that of the pressed juice. (This can be explained by the lower amount of ruptured cells present.)

Increasing extraction temperature leads to enrichment of the juice in non-sugar substances. Raising the extraction temperature to 60 °C the total sugar content will be lower and the non-sugar substance higher, related to total dry matter, than in the pressed juice.

In juice extracted at lower temperature the proportion of fructose and glucose is higher, too. With increasing extraction temperature the proportion of these sugars in relation to total dry matter decreases, too.

As seen from the results of measurements the saccharose becomes inverted while the fructose formed continues to break down. Glucose, on the other hand, is more stable at the pH of the apple juice. Thus, with increasing temperature, the inversion of saccharose becomes more and more intense and consequently the glucose forms an increasing proportion of the total sugar content.

As it can be seen in Figs. 1 and 2, the finding valid for the average at the average temperature (for Jonathan 56.3 °C, for Starking 56.8 °C) of the extraction experiments, is true, namely that extracted juices are poorer in saccharose and richer in reducing sugars than the pressed juices. Proportions, however, change with temperature because of the difference in temperature dependence of saccharose inversion and fructose or glucose decomposition.

The summarized results show that the pectin content in extracted juices is significantly higher than in pressed juices. This is because the enzyme activity inherent in the apple juice becomes substantially lower above 50 °C. While in the course of pressed juice production enzyme activity is enhanced already in the mash, in the apple slices prepared for extraction this is much less possible and if slices are soon extracted at about 57 °C temperature enzymes become completely inactivated. If, however, the extraction temperature is raised, enzymatic decomposition is replaced by pectin hydrolysis, which becomes intensive. If the pectin content of juice extracted at higher temperature is compared to the pectin content of the control sample the difference between the two will be lower than that of juice extracted at lower temperature. Namely, the pectin content is determined as Ca-pectate and the short chain pectin molecules do not precipitate any more with Ca^{++} ion and thus cannot be detected by chemical analysis.

The colour of juices (absorbance at 425 nm of the juice clarified by centrifugation) becomes darker with increasing extraction temperature.

The juice of Jonathan apples extracted at 50 °C is substantially lighter than the pressed juice. This advantage, however, diminishes gradually with higher temperatures, although the colour of the juice extracted at 57 °C is still significantly lighter than that of the pressed juice.

Only the extracted juice of Golden Delicious variety was darker on the average than the pressed juice. The higher was the extraction temperature the greater became this difference to the advantage of the pressed juice.

It is worth noting that all the above reactions are more temperature dependent with Jonathan apples than with Starking apples, probably due to the lower pH of Jonathan juice.

2.3. Sensory evaluation of pressed and extracted juices

The juices obtained in parallel by pressing and extraction, were frozen. Prior to sensory evaluation they were thawed, roughly filtered, uniformly set at 9.0 g per 100 cm^3 water soluble dry matter content and 15 °C temperature. They were tested in their natural condition by a panel of 8 experienced members.

(The samples obtained from the same fruit variety were unified, thus, finally 3 extracted and 3 pressed juices were compared.)

The aim was to see whether panel members could differenciate between sample pairs prepared in two different ways, and if so, which of the two samples was better? The triangular test was found the most suitable to judge the identity or dissimilarity of samples. The principle of the test method is that each panel member is given 3 samples at a time of which two are identical and the 3rd different. The task of the panel member is to establish which are the two identical samples and which one is different. The triangular test was complemented by the question whether the different sample was better or worse than the two identical samples.

In the case of the Jonathan apples all the 8 panel members recognized the difference by taste, smell and colour between the pressed and the extracted juice. All the panel members found the colour of the extracted juice lighter, therefore better. The taste and smell of the extracted juice was found to be better by 5 of the 8 panel members. This proves the extracted juice to be statistically better ($P = 5\%$) in the case of this apple variety.

In the case of Starking variety the difference was recognised in taste and smell between the two kinds of juice. Six panel members out of the 8 found the pressed juice better than the extracted juice ($P = 5\%$). The colour of the two juices was found to be identical.

In the case of Golden Delicious variety the difference was discovered only in the taste of the two samples, in smell and colour it was not observed. Six out of the 8 panel members found the taste of the pressed juice better, this shows the difference to be significant ($P = 5\%$).

Thus, the sensory evaluation proved that the juices, obtained by two different methods differ in their sensory properties. It was however, not decided unanimously which of the two juices was better, the opinion varied with apple variety. Similar conclusions can be drown from data published in related literature.

From the point-of-view of the extraction technology Jonathan variety proved to be superior to the other varieties by sensory test. The sugar to acid ratio and the relatively high acid content in this variety ensured that in spite of the slight loss of acid during extraction it did not become insipid of taste. The other two varieties are originally poorer in acid and the additional loss in acid due to extraction technology causes then to be unsuitable for concentrate production. Beside the harmonious taste the light colour of extracted Jonathan juice is a favourable property.

This study has shown that in the controversy about the two different manufacturing technologies of apple juice the variety plays an important role.

Literature

ANON. (1979): Extraction/Diffusion — in der Bundesrepublik Deutschland bei der Herstellung und Import von Fruchtsäften bisher nicht zugelassen. *Flüss. Obst, 46*, 367–368.

BINKLEY, C. R. & WILEY, R. C. (1978): Continuous diffusion — extraction method to produce apple juice. *J. Fd Sci., 43*, 1019–1023.

CONCEN, D., ÖZMEN, T. & POSSMANN, P. (1977): Extraktion quo vadis? *Flüss. Obst, 44*, 122–132.

DOUSSE, R., GIRARD, J. M. & EMCH, F. (1977): L'extraction solide-liquide par diffusion de jus de pomme. Etude des influences de la température du systhème. *Inds. aliment agric., 94*, 1283–1287.

GHERARDI, S. (1978): La diffusione una nuova tecnica per l'estrazione dei succhi di frutta. *Industria Conserve, 53*, 20–24.

GIERSCHNER, K., HAUG, M. & WIRNER, H. (1978): Technik und offene Probleme der Extraktion, einem neueren Verfahren zur Gewinnung von Säften aus Äpfeln und anderen Früchten. *Dt. LebensmittelRdsch., 74*, 438–443.

GOLDBACH, M., HAUG, M., GIERSCHNER, K. & WIRNER, H. (1978): Vergleich analytischer Kennzahlen von Apfelsäften, die durch Auspressen und durch Extraction im Industriemaßstab gewonnen wurde. *Flüss. Obst, 45*, 322–328.

HUNGARIAN STANDARD (1971): *Tartósított élelmiszerek. Szárazanyag és víztartalom meghatározás.* (Conserved foods. Determination of dry substance and of water content.) MSz 3607/1–71.

HUNGARIAN STANDARD (1974a): *Tartósított élelmiszerek. Cukortartalom meghatározása.* (Canned foodstuffs. Determination of sugar content.) MSz 3625–74.

HUNGARIAN STANDARD (1974b): *Tartósított élelmiszerek összes savtartalmának meghatározása.* (Determination of total acid content in preserved foods.) MSz 3619–74.

HUNGARIAN STANDARD (1983): *Tartósított élelmiszerek összes hamutartalmának és hamulúgosságának meghatározása.* (Processed foods. Determination of total ash and alcalinity of ash.) MSz 3610–83.

HAUG, M. & GIERSCHNER, K. (1979): Auswirkung unterschiedlicher Herstellungsverfahren (Auspressen, Extraction) auf die phenolischen Verbindungen von Apfelsäften. *Dt. LebensmittelRdsch, 75*, 248–253; 274–276.

KARDOS, E. (1962): *Gyümölcs- és zöldséglevek, üdítőitalok.* (Fruit and vegetable juices, soft drinks.) Műszaki Könyvkiadó, Budapest.

LAURSEN, J. (1974): Herstellung von Apfelsaft, mit der DDS Diffusion. *Flüss. Obst, 41*, 284–286.

LÜTHI, H. R. & GLUNK, U. (1974): Gewinnung von Apfelsaft durch kontinuierliche Extraction. *Flüss. Obst, 41*, 498–505.

LÜTHI, H. R. & GLUNK, U. (1979): Extraktion und kontinuierliche Schönung. 3. Entwicklung der Extraktion von Apfelsaft und Betriebserfahrungen. *Flüss. Obst, 46*, 111–113; 116–120.

OTT, J., GÁL, F. & PEÁK, L. (1960): Almalényerés diffúziós úton. (Apple juice production by diffusion.) *Konzerv Paprikaipar*, 156–163.

PÁTKAI, GY. (1986): Hazai almafajták alkalmassága extrakciós lényerés céljára. (The suitability of Hungarian apple varieties for juice production by extraction.) Thesis, Budapest.

PEDEN, D. (1976): Über die Cider und Apfelsaftherstellung in England. *Flüss. Obst, 43*, 182, 187–188.

POSSMANN, PH., RÖLL, S., SEYFFARTH, K. & WUCHERPFENNIG, K. (1975): Über vergleichende Untersuchungen von Entsaftungsverfahren am Beispiel von Äpfeln. *Flüss. Obst, 42*, 408–410.

SCHOBINGER, U., DOUSSE, R., DÜRR, P. & TANNER, H. (1978): Vergleichende Untersuchungen von Preßsaft, Diffusionssaft und Tresterextrakt aus Äpfeln. *Flüss. Obst, 45*, 210–216.

SINGLETON, V. L. & ROSSI, J. (1970): Colorimetry of total phenolics with phosphomolibdic-phosphotungstic reagents. *Am. J. Enol. Vitic., 21*, 144–158.

TRENKLE, K. (1984): Weltweite Standardisierung von Fruchtsäften und Nektaren. *Flüss. Obst, 51*, 282–283.

WUCHERPFENNIG, K., POSSMAN, P. & ÖZMEN, T. (1976): Über der Einsatz der Extraktion. *Flüss. Obst, 43*, 119–121.

WUCHERPFENNIG, K. & POSSMANN, P. (1979): Zur Entwicklung der Entsaftungsverfahren. *Flüss. Obst, 49*, 282–289.

Acta Alimentaria, Vol. 18 (2), pp. 167—176 (1989)

COMPARISON OF RHEOLOGICAL, CHEMICAL AND SENSORY PROPERTIES OF PULPED TOMATOES PRESERVED BY VARIOUS TECHNIQUES

L. JIMENEZ, J. L. FERRER and A. CHICA

Departamento de Ingenieria Quimica, Facultad de Ciencias,
Universidad de Córdoba, C/ San Alberto Magno s/n, 14004 — Córdoba. Spain

(Received: 23 December 1987; accepted: 27 September 1988)

The rheological, chemical and sensory characteristics of pulped tomatoes preserved by freezing, freeze-drying and pasteurization are compared.

The values of the yield stress and the consistency index of the products are in this order: fresh, pasteurized, frozen and freeze-dried. The values of the flow behaviour index, in the forward and return runs, have been determined for all the products.

In order to quantify the time-dependence, the Weltman and two proposed equations are used. While some parameters of these equations vary following the previous sequence, others, which reflect the structural rupture rate, vary following this sequence: fresh, freeze-dried, pasteurized, and frozen.

The sugar, pectin, soluble solid and insoluble solid contents and pH do not vary from one product to another. However, the vitamin C content is 40% lower for the pasteurized product.

Through the use of sensory tests which compare the colour, odour, flavour and texture of the products, it can be deduced that the best process is freezing and the worst is freeze-drying.

Keywords: pulped tomatoes, rheology, chemical properties, freezing, freeze-drying, pasteurization, sensory characteristics

Tomato (*Licopersicum esculentien* L.) crops are usually much more abundant than those of other horticultural products. The overall production of tomatoes in Spain throughout 1985 was 3 174 000 tons and thus significantly exceeded that of onions (1 244 000 tons), melons (784 000 tons) or paprikas (736 000 tons) (SPANISH MINISTRY OF AGRICULTURE, 1986).

Most of the tomato crop is consumed fresh; yet, the consumption of preserved tomato grew from 20% to 26% between 1983 and 1985 (SPANISH MINISTRY OF AGRICULTURE, 1986).

One simple method of preserving pulped tomatoes could be that of pasteurization which, in contrast to freezing, does not require the use of much energy for its preservation. Pasteurization also requires less investment and has lower operation costs than another preservation method, that of freeze-drying.

In this paper, pasteurized, frozen and freeze-dried products are compared with the fresh product with respect to their rheological, chemical, and sensory characteristics.

1. Materials and methods

The material used was pulped tomatoes containing 4.31% total solids and solid particles less than 0.1 mm in diameter.

Freezing takes place in a conventional freezer whose temperature reaches −40 °C. In cylindric glass receptacles of 30 mm diameter, samples are frozen from 20 °C to −20 °C in a period of 65 minutes.

The freeze-drying system consists of a drying chamber, a vapour condensor and vacuum system. It is described in another paper (JIMENEZ et al., 1978). In 10 mm height trays, samples are frozen till −20 °C. After that, they are freeze-dried under a pressure of 93.3 Pa and a temperature of 50 °C.

The pasteurizer used consists of a heat exchanger in a thermostatic bath, where the pasteurization takes place, and another heat exchanger, from which the product exits both pasteurized and cold. The product was pasteurized at 100 °C for 60 s and subsequently cooled at 25 °C for 5 s.

A coaxial cylinder viscometer with a rotor of 35 mm diameter by 65 mm height and coaxial cup of 38 mm diameter, was used. The cylinder could rotate at 8 different speeds. A more detailed description of the viscometer and its operation can be found in another paper (JIMENEZ & LOPEZ, 1983).

Vitamin C was determined by the 2,6-dichloroindophenol method (ALEMANY & FONT, 1983), while sugars and pectins were analyzed by the Lane and Eynon method (PEARSON, 1973) and the Rowse and Atkins method (ROYO et al., 1975), respectively. Soluble solids were determined by the AOAC's 20013–20014 methods and insoluble ones were analyzed refractometrically (HART & FISHER, 1971). The pH was measured with a pH-meter, following the method specified by PEARSON (1973).

The sensory properties were evaluated by a panel of tasters who compared the processed samples with the fresh ones. The panel members were familiarized with the characteristics of the fresh pulped tomatoes. They were selected if the results of ten different tests had a reproducibility of 90%.

Sensory tests value the colour, odour, flavour and texture of the products. The products are classified by ordering them (number 1 to 4) according to their decreasing acceptability, for which the comparison pattern is that of the fresh product (number 1).

2. Results

Figures 1 and 2 show the experimental results of the shear stress τ, versus shear rate, γ, of the products, at 20 °C, in the successive forward and return runs (increase: 3 minutes and decrease: 3 minutes, respectively) of the spin velocity of the viscometer. The hysteresis cycles, which appear in

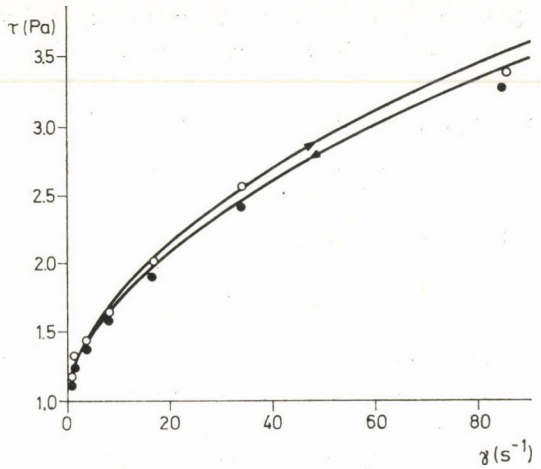


Fig. 1. Shear stress (τ, Pa) versus shear rate (γ, s^{-1}) for fresh pulped tomatoes, measured at 20 °C. o: Forward run; ●: return run

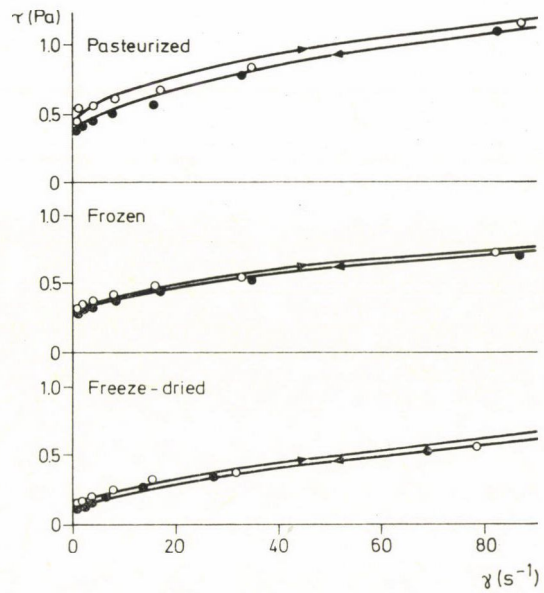


Fig. 2. Shear stress (τ, Pa) versus shear rate (γ, s^{-1}) for pasteurized, frozen and freeze-dried pulped tomatoes, measured at 20 °C. o: Forward run; ●: return run

Figs. 1 and 2, are closed when $\gamma = 170\ s^{-1}$ and the values of τ are 4.41, 1.03, 0.92; $y = 1.64$ Pa for fresh, frozen, freeze-dried, and pasteurized product, respectively. Other four similar experiments have been carried out with every product; it was found that the deviations of τ values, in relation to its values which are shown in Figs. 1 and 2, are always below 10%.

By observing these curves, it can be deduced qualitatively that all the products are pseudoplastics and have a yield stress and a thixotropic time dependence.

Modified Casson equation (JIMENEZ & DIAZ, 1986):

$$\tau^{0.5}(1 + S) = 2(\tau_0)^{0.5} + K_0 (1 + S) \gamma^{0.5} \quad (1)$$

and the Herschel and Bulkley model:

$$\tau = \tau_0 + K \gamma^n \quad (2)$$

(where τ is the shear stress, γ the shear rate, τ_0 the yield stress, S the relationship between the radii of the rotor of the viscometer and its coaxial cup, K the consistency index, n the flow behaviour index and K_0 a characteristic constant) were used to quantify the rheological behaviour of the products.

With the adjustment of the experimental data τ and γ to these equations, the values of τ_0, K and n shown in Table 1 are obtained. The correlation coefficients are always 0.99.

As can be seen, τ_0 decreases in the order: fresh, pasteurized, frozen, and freeze-dried. The decreases of τ_0 for the pasteurized, frozen and freeze-dried products with respect to the fresh product indicate that the preservation process that most distorts the products is freeze-drying and that which least distorts them is pasteurization.

The same is indicated in relation to the consistency index, as shown by the decreases of the values of this parameter for the various processed products compared to the fresh product.

The value of n is smaller for the frozen and pasteurized products and greater for the freeze-dried product, in comparison to the fresh product. This indicates that the frozen and pasteurized products are made more pseudoplastic while the freeze-dried product loses pseudoplasticity. Given that average difference of the flow behaviour index, compared to the fresh product, is of the order of 10%, it can be considered that the pseudoplasticity of the

Table 1

Yield stress (τ), consistency index (K) *and flow behaviour index* (n) *for fresh, frozen, freeze-dried and pasteurized pulped tomatoes*

Pulped tomatoes	Forward run			Return run		
	$\tau_0 10^{-2}$	$K 10^{-2}$	n	$\tau_0 10^{-2}$	$K 10^{-2}$	n
Fresh	100.15	22.31	0.55	93.73	22.65	0.54
Frozen	25.47	5.91	0.48	23.02	5.79	0.49
Freeze-dried	10.01	3.78	0.60	7.90	3.38	0.63
Pasteurized	37.82	9.92	0.47	27.79	9.70	0.48

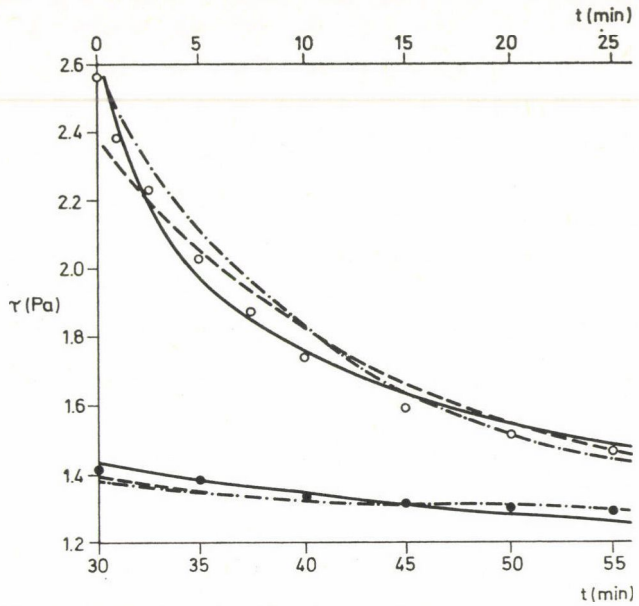


Fig. 3. Shear stress (τ, Pa) versus time (t, min) for fresh pulped tomatoes, measured at 20 r. p. m. and 20 °C. ○: Upper axis; ●: under axis; ——: Weltman; ----: equation 4; -·-·-: equation 5

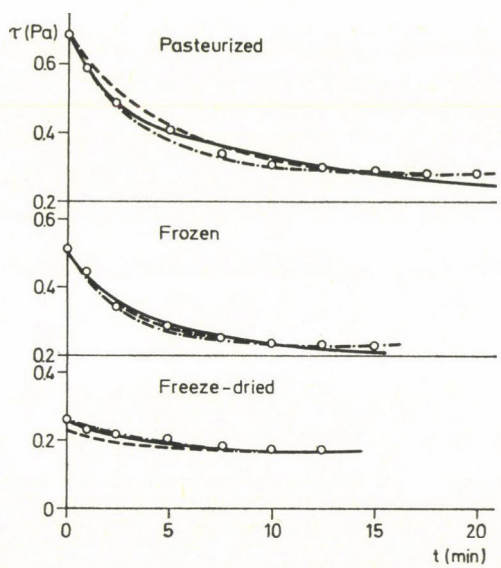


Fig. 4. Shear stress (τ, Pa) versus time (t, min) for pasteurized, frozen and freeze-dried pulped tomatoes, measured at 20 r. p. m. and 20 °C. ——: Weltman, ----: equation 4, -·-·-: equation 5

pulped tomatoes is practically uninfluenced by the type of process used in its preservation.

Simulation of the experimental results of shear stress by applying the Harschel and Bulkley equation show that the results are reproduced with errors of less than 10% in the worst case. The calculated values of τ as a function of γ are shown in Figs. 1 and 2 by a continuous line.

Figures 3 and 4 represent the experimental results of the shear stress as a function of time, at 20 r.p.m. and 20 °C, of all the products. Four similar experiments have been carried out with every product and it was found that the deviations of τ values in relation to the values shown in Figs. 3 and 4, are always below 10%.

Qualitatively, it can be deduced that these products, being thixotropic, are dependent upon time.

In order to quantify this dependence on time, the following equations are used:

WELTMAN (1943):

$$\tau = A - B \ln t \tag{3}$$

Proposed:

$$(\tau - \tau_e)^{1-m} = A_1 - A_2 t \tag{4}$$

Proposed:

$$\ln \{(\tau_i - \tau_e)/(\tau - \tau_e)\} = C\tau_i t (\tau_i - \tau_e) \tag{5}$$

where t is time, τ_i and τ_e are the shear stress at time zero and infinity, A and A_1 are constants that reflect the shear stress with which the shearing had been started, B is the time coefficient of thixotropic rupture, A_2 and C are constants that reflect the structural rupture rate, and m is a characteristic constant.

Equation 4 represents a model involving a rheological equation modified by introducing a time-dependent structural parameter, λ, which ranges from an initial value of unity for zero shear time to an equilibrium value, λ, which is less than unity (TIU & BOGER, 1974):

$$\tau = \lambda(\tau_0 + K\gamma^n) \tag{6}$$

as well as another equation accounting for the rate of change of the structural parameter (PETRELLIS & FLUMERFEITT, 1973);

$$d\lambda/dt = -K'(\lambda - \lambda_e)^m \tag{7}$$

where K' is the rate constant and m is an order constant. Both, λ and K', are dependent on the shearing rate.

A suitable combination of the above expressions yields:

$$(\tau - \tau_e)^{1-m} = (\tau_i - \tau_e)^{1-m} - [K'(1 - m)/(\tau_0 + K\gamma_0^n)^{m-1}] t \tag{8}$$

Equation 8 can be simplified to 4.

Equation 5 represents a model assuming the product to have a complex structure A reversibly evolving to a simpler structure, B through shearing:

$$A \rightleftharpoons B$$

Thus, the rate of disappearance of A can be expressed as:

$$dC_A/dt = -K_i(C_A)^{m'} f(\gamma) \tag{9}$$

where C_A is the concentration or fraction of A, K_i is the rate constant of disappearance of A, m' is the order in C_A and $f(\gamma)$ is a function of the shearing rate.

On the other hand, the shear stress required to give rise to a given shear rate depends on the fraction of A and on the shear rate itself:

$$\gamma = K_2 C_A f'(\gamma) \tag{10}$$

A suitable combination of equations 9 and 10 yields:

$$d\tau/dt = -K_i f(\gamma)\tau^{m'}/K_2 f'(\gamma)^{m'-1} = -C\tau^{m'} \tag{11}$$

where C can be considered a rate constant related to the destruction of the product structure.

As B can in turn yield A,

$$d\tau/dt = K_3 (\tau_i - \tau)^{m'} \tag{12}$$

where K_3 is the rate constant of recombination of B.

By adding up equations 11 and 12 and rearranging, we obtain:

$$d\tau/dt = C\{(\tau_e/(\tau_i - \tau_e))^{m'} (\tau_i - \tau)^{m'} - \tau^{m'}\} \tag{13}$$

If $m' = i$, the integration of equation 13 between τ_i and τ for $t = 0$ and $t = t$ yields equation 5.

Upon adjustment of the experimental data of τ and t to equations 3, 4 and 5, the values of the parameters that appear in Table 2 are obtained. In this table are also found the values of A_1 and A_2 of equation 4 when m has an average value of 0.85. The correlation coefficients of the adjustment of the experimental data to equations 3, 4 and 5 are always 0.99.

As can be observed, the values of A, B and A_1 of the products decrease in this order: fresh, pasteurized, frozen, and freeze-dried. As it was mentioned before, it happened in the same way with τ_0 and K parameters in Table 1. However, the parameters related to the structural rupture rate (A_2 and C) increase in this product order: fresh, freeze-dried, pasteurized and frozen.

From the above, it can be deduced that time dependency is valued differently in the two groups of parameters considered.

Table 2

Parameter values of the Weltman and proposed equations for fresh, frozen, freeze-dried, and pasteurized pulped tomatoes

Parameter	Fresh	Frozen	Freeze-dried	Pasteurized
$A\ 10^{-3}$	2445.88	426.52	234.24	586.80
$B\ 10^{-3}$	299.04	83.16	24.22	112.67
$A_1 10^{-3}$	1010.91	823.08	613.97	876.11
$A_2 10^{-3}$	9.64	36.53	25.39	26.18
m	0.85	0.85	0.84	0.86
$C\ 10^{-3}$	42.10	213.25	83.44	165.31
$A_1 10^{-3}$	1010.91	823.08	681.12	866.92
$A_2 10^{-3}$	9.64	36.53	23.05	27.28

In the last values of A_1 and A_2 it is considered that $m = 0.85$

Table 3

Results of the chemical analyses applied to fresh, frozen, freeze-dried, and pasteurized products

Analyses	Fresh		Frozen		Freeze-dried		Pasteurized	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Vitamin C ($\mu g\ cm^{-3}$)	54.6	1.3	49.1	1.4	56.5	1.5	31.3	1.3
Sugars ($mg\ cm^{-3}$)	27.9	0.8	26.7	1.0	27.2	0.9	28.5	0.8
Soluble pectin (%)	0.55	0.03	0.42	0.04	0.40	0.03	0.53	0.04
Insoluble pectin (%)	0.74	0.05	0.86	0.04	0.90	0.05	0.80	0.06
Protopectins (%)	0.40	0.04	0.40	0.04	0.53	0.05	0.40	0.04
pH	4.17	0.06	4.11	0.09	4.11	0.06	4.12	0.07
Soluble solids (%)	3.21	0.06	3.20	0.05	3.16	0.04	3.20	0.05
Insoluble solids (%	1.08	0.03	1.07	0.03	0.96	0.04	1.10	0.04

Finally, upon simulation of the results of using equations 3, 4 and 5, it is found that they can be reproduced with errors of less than 5% for all assayed product. The τ values, which have been calculated by the already mentioned equations, are shown in Figs. 1 and 2 as functions of γ values.

For five experiments, the average values of the composition and pH of the four products is given in Table 3. The standard deviations (σ) also appear there.

It can be deduced that the preservation processes do not influence reducing sugars, pectins, pH, soluble solids, or insoluble solids. On the other hand, the vitamin C content decreases in the pasteurized product by more than 40%.

Table 4

Classifications (from 1 to 4; from best to worst) given by the tasters, for the different products: fresh (F), *frozen* (C), *freeze-dried* (L), *and pasteurized* (P)

Characteristic	Product	Number of panelists												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Colour	*F*	3	3	1	1	3	1	1	1	1	1	3	1	1
	C	1	1	3	2	2	3	2	2	2	3	1	2	2
	L	4	2	4	3	1	4	4	4	4	4	4	4	3
	P	2	4	2	4	4	2	3	3	3	2	2	3	4
Odour	*F*	1	1	1	1	1	2	2	2	1	1	2	1	1
	C	2	3	2	2	4	3	1	1	2	4	1	2	3
	L	3	4	3	4	2	4	3	4	3	2	4	4	4
	P	4	2	4	3	3	1	4	3	4	3	3	3	2
Flavour	*F*	4	1	3	1	1	1	1	2	1	1	4	1	2
	C	1	4	1	3	4	2	4	1	4	4	1	4	1
	L	3	2	4	2	3	3	3	4	3	2	3	3	3
	P	2	3	2	4	2	4	2	3	2	3	2	2	4
Texture	*F*	4	1	1	2	2	2	3	1	1	2	2	1	3
	C	2	3	4	1	3	3	1	3	3	4	3	3	1
	L	1	4	3	4	1	1	4	4	4	1	1	4	4
	P	3	2	2	3	4	4	2	2	2	3	4	2	2

In Table 4 the results of the sensory tests are presented.

Through the application of the Kramer and Twigg tables (COSTELL & DURAN, 1982) it can be deduced that there are no significant differences of colour, odour or flavour at the 1% significance level. The fresh product is that which has the best characteristics, compared to the freeze-dried which has the worst characteristics.

Comparing the frozen and freeze-dried products, it can be seen that at the 5% significance level, there are differences in colour and odour but at the 1% significance level only in colour.

Also, it is verified that at the 5% and 1% significance levels, there are neither significant differences between the frozen and pasteurized products, nor between the pasteurized and freeze-dried products.

Upon comparison of the fresh and frozen products it is deduced that there are no significant differences for any of the characteristics. If the fresh and pasteurized products are compared, there are differences of colour and odour at the 5% significance level but only of odour at the 1% level. Lastly, comparing the fresh and freeze-dried products, it can be seen that at the 5% significance level, there are differences in colour, odour and flavour and, at the 1% level, only in odour.

3. Conclusions

Seen from the point of view of the values of the parameters of the Herschel and Bulkley equation, the product which deviates most from the fresh is the freeze-dried product and that which least deviates is the pasteurized product. The values of yield stress vary between 100.75×10^{-2} and 7.90×10^{-2} Pa and those of the consistency index between 22.65×10^{-2} and 3.38×10^{-2}. The values of the flow behaviour index vary between 0.48 for the frozen product and 0.60 for the freeze-dried product in the forward run. In the return run, they vary between 0.48 for the pasteurized product and 0.63 for the freeze-dried product.

The same conclusion is arrived at upon analysis of the values of the parameters A, B, and A_1 of the equations which indicate time dependence. However, the values of the parameters A_2 and C indicate that the freeze-dried product is that which approximates closest the fresh product and the frozen product that which least approximates it.

It is seen from the results of the chemical analyses that the freezing and freeze-drying processes yield products similar to the fresh product while the pasteurized process destroys vitamin C.

Finally, it is deduced from the sensory tests that the product which is most similar to the fresh product is the frozen product and the most different is the freeze-dried product.

Literature

ALEMANY, M. & FONT, S. (1983): *Prácticas de Bioquímica*. Alhambra, Madrid, pp. 96–99.

COSTELL, E. & DURAN, L. (1982): El analisis sensorial en el control de calidad de los alimentos. — Part III. Planificacion, seleccion de jueces y diseño estadístico. *Rev. Agroquim. Tecnol. Aliment.*, *21*, 454–470.

HART, F. L. & FISHER, H. J. (1971): *Analisis moderno de los alimentos*. Acribia, Zaragoza, pp. 293–298.

JIMENEZ, L. & DIAZ, R. M. (1986): Estudio reologico de los componentes de la mezcla aceite de oliva-huevo-zumo de tomate. *Afinidad*, *43*, 311–320.

JIMENEZ, L. GONZALEZ, E. & ROMERO, A. (1978): Estudio cinético de la liofilización de la crema de leche. *An. Quim.*, *74*, 134–140.

JIMENEZ, L. & LOPEZ, A. (1983): Viscosimetro rotatorio para medidas reológicas de productos liquidos. *Afinidad*, *40*, 448–452.

PEARSON, D. (1973): *Laboratory techniques in food analysis*. Butterworths, London, pp. 249–251.

PETRELLIS, N. C. & FLUMERFELTT, R. W. (1973): Rheological behaviour of shear degradable oils: kinetic and equilibrium properties. *Can. J. chem. Engng.*, *51*, 291–297.

ROYO, C., MIRALLES, M. C. & CLARAMUNT, P. (1975): Preparación de corteza seca de naranja para la obtención de pectina a partir de variedades cultivades en España. *Rev. Agroquim Tecnol. Aliment.*, *15*, 539–546.

SPANISH MINISTRY OF AGRICULTURE (1986): *Anuario de estadística agrario*. Madrid, pp. 548–580.

TIU, C. & BOGER, D. V. (1974): Complete rheological characterization of time-dependent food products. *J. Texture Stud.*, *5*, 329–338.

WELTMAN, R. N. (1943): Breakdown of thixotropic structure as function of time. *J. appl. Phys.*, *14*, 343–350.

Acta Alimentaria, Vol. 18 (2), pp. 177—192 (1989)

THE EFFECT OF DATE SYRUP CONCENTRATION ON GROWTH RATE, PROTEIN, RNA CONTENT AND PROTEASE ACTIVITY OF *S. CEREVISIAE*, *C. GUILLIERMONDII* AND *R. GLUTINIS*

MOHAMED KASSIM MUSTAFA and A. HALÁSZ

Central Food Research Institute, H-1022 Budapest, Herman O. út 15.
Hungary

(Received: 20 January 1988; accepted: 30 August 1988)

With increasing date syrup content of the fermentation medium, growth rate of *S. cerevisiae*, *C. guilliermondii* and *R. glutinis* increased. Protein content of *S. cerevisiae* increased in the range of 0.1 to 0.3% date syrup concentration. The whole synthesized protein increases at higher date concentration for each strain. RNA content of the investigated yeast strains varied with date syrup concentration and strain as well. For *S. cerevisiae* increase in carbon source concentration caused a gradual increase in RNA. *C. guilliermondii* seemed to be independent of date syrup concentration in this respect. The proteinase activity at the end of fermentation was highest in *S. cerevisiae* with maximum value at 0.5% date syrup concentration. *C. guilliermondii* showed similar variation in protease activity as *S. cerevisiae*, but the absolute values were significantly lower in each case. Our experiments showed that date syrup is a good substrate for yeast strains of different genera. For biomass production *C. guilliermondii* and *R. glutinis* gave much better results in cell concentration than *S. cerevisiae*.

Keywords: date syrup, protease activity, RNA content

Dates, the fruit of *Phoenix dactylifera*, are the most important staple food in some countries of the world as e.g. Iraq. Dates are divided into three types according to solids content which consists mostly of sugars. Total sugar content in dates varies from 47% to 85%, depending on the variety (KAMEL, 1979; MOHAMED & AHMED, 1981; SACHDE et al., 1981). The main components of the carbohydrate content are: Sucrose, glucose, fructose, and a very small amount of polysaccharides. The sugars can be obtained from dates by water extraction. Dates also contain nitrogeneous substance and minerals (P, Ca, K, Na, Fe) (SAKURADA, 1966; AL-RAWI et al., 1967). Growth factors in dates are of great importance considering microbial fermentation (KAMEL, 1979). Usually, the date syrup is produced from the cheap varieties which contain a high percentage of sugars. In several studies date juice is mentioned as an interesting substrate for utilization in SCP production and wine industry (SALEM & HEGAZI, 1971).

The physiological properties of yeast cells are strongly influenced by the culture medium. SAKURADA (1966) found that yeast grown on glucose has low respiratory activity, and the cells multiply vigorously in glucose

medium as compared to ethanol medium. Two stages could be observed in the cell state. The duration and efficiency of the single cell state is dependent on growth limitation and the carbon source (HEINRITZ et al., 1983).

Today, there is a great interest in obtaining a new source for microbial proteinases as these enzymes are of increasing importance in the food industry. The relative commercial importance of microbial proteinases as compared to other enzymes can be appreciated from their estimated world sales which amount to 40% of total enzyme sales for industrial purposes (WILLIAM, 1983).

Intracellular proteinases of yeast have been known for a long time. According to recent work on the proteinases A and B (endopeptidases) and carboxypeptidase C they are localized in the vacuoles (HOLZER, 1977; HOLZER, 1975; LENNEY, 1955). Eight proteases have been characterized in *Saccharomyces cerevisiae*, two endoproteinases, two carboxypeptidases, three aminopeptidases and one dipeptidase (ACHSTETTER et al., 1982; HINZE et al., 1975; WOLF, 1982).

However, little is known on proteinases in Candida and Rhodotorula strains and the influence of fermentation parameters on the proteolytic enzyme activity.

The general functions of intracellular proteinases in microorganisms are the degradation of proteins in general, protein turnover, and degradation of non-functional ("nonsense") proteins (HOLZER, 1977; WILLIAM, 1983). HOLZER (1975) found that proteinases participate in the regulation of metabolism by degrading enzymes under certain physiological conditions.

The present study was undertaken in order to establish:

— The relationship between carbon source and proteinase activity of yeasts belonging to three different genera.

— The effect of the carbon source on RNA and protein content in yeasts.

1. Materials and methods

1.1. Utilized media

1.1.1. Yeast extract medium. Glucose 10 g l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.1 g l^{-1}, KH_2PO_4 0.14 g l^{-1}, $Na_2HPO_4 \cdot 12H_2O$ 0.8 g l^{-1}, NaCl 1.0 g l^{-1}, $(NH_4)_2SO_4$ 4.0 g l^{-1}, yeast extract 5.0 g l^{-1}, and agar 20 g l^{-1} completed to 1 l with distilled water, pH adjusted to 4.5.

1.1.2. Synthetic medium. $MgSO_4 \cdot 7H_2O$ 0.1 g l^{-1}, KH_2PO_4 0.14 g l^{-1}, $Na_2HPO_4 \cdot 12H_2O$ 0.7-g l^{-1}, NaCl 1.0 g l^{-1}, $(NH_4)_2SO_4$ 4.0 g l^{-1}, glucose 5 g l^{-1}, yeast extract 5.0 g l^{-1} completed to 1 l with distilled water, pH adjusted to 4.5.

1.1.3. Date syrup medium. The date syrup was obtained from the State Enterprise for Canning in Iraq. Dry solids content of date syrup was 68–70%.

This concentrate was diluted with distilled water to 25% final concentration as determined by a refractometer. This solution was dispensed into 250 cm^3 conical flasks. The flasks were sealed with cotton plugs and autoclaved at 105 °C for 20 min.

1.2. Investigated yeast strains

Three strains of yeast were used in this study.

— *Saccharomyces cerevisiae:* A commercial strain of baker's yeast produced by the Yeast Factory Budafok, Budapest.

— *Rhodotorula glutinis* 803 obtained from Department of Microbiology, József Attila University, Szeged.

— *Candida guilliermondii* 810 obtained from Department of Microbiology, József Attila University, Szeged.

1.3. Yeast propagation techniques

1.3.1. Surface culture in Petri dishes. The yeast cultures were grown on a glucose–yeast-extract medium at 32 °C for 24 h.

1.3.2. Shaken culture. The inocula were prepared by transfering the cultures from the Petri dishes into 500 cm^3 Erlenmeyer flasks which contained 200 cm^3 synthetic medium. The flasks were shaken in a rotary shaker at 330 r.p.m. for 24 h at 30 $\pm$ 1 °C. (A cell suspension of 0.21% cell solids was propagated in a column fermentor.)

1.3.3. Column fermentor. Jacketed glass columns of 500 cm^3 were used. The date syrup medium contained date syrup and 5 g l^{-1} yeast extract, made up to 500 cm^3 volume with sterilized distilled water. Five concentrations of date syrup 0.1%, 0.3%, 0.5%, 0.8%, 1.0% were used. For control purposes, synthetic medium (para 1.1.2.) with only 0.5% glucose content was used.

Filtered air was bubbled from the bottom of the column through a sintered glass filter. Temperature was maintained at 30 °C and air flow at 600 dm^3 h^{-1}. The pH value of the medium was 4.5–5.0.

Fermentation was started with a cell suspension propagated in shaken culture for 24 h to reach a starting yeast concentration of 0.20% dry weight.

1.4. Determination of proteolytic activity

Proteolytic activity was determined by ANSON (1939) method. To release the enzyme, the cell wall was destroyed by ultra sonication.

Preparation of cell free extract yeast cultures were harvested by centrifugation, washed twice with distilled water, suspended in 40 cm^3 distilled water, dispensed in an ice bath and sonicated three times with the Labsonic

type 1510 sonicator (3 min at 300 W). Half cm^3 of TRITON 100 was added to the sample and this was transferred to a shaker for 1 h at 32 °C. The cell-free extract was collected by centrifugation.

Preparation of substrate: 4 g hemoglobin ground with 70 g urea, 16 cm^3 1 *N* NaOH and 800 cm^3 distilled water were added. The hemoglobin solution was agitated for 30 min with a magnetic stirrer, then it was adjusted to pH 5 with acetic acid and filled up to 200 cm^3.

The reaction: 5 cm^3 portions of hemoglobin solution were filled into 25 cm^3 Erlenmeyer flasks which were shaken in a water bath at 40 °C. One cm^3 of cell-free extract was added to each flask. The reaction was stopped after 60 min by adding 10 cm^3 of 0.3 mol l^{-1} of TCA. The solution was clarified by centrifugation at 3000 r.p.m. for 15 min. Five cm^3 of clear solution and 10 cm^3 Merck Folin reagent (1 : 2 Folin reagent to distilled water) were added. The absorbance was measured at 660 nm in a spectrophotometer against blank (5 cm^3 of hemoglobin solution was shaken at 40 °C for 1 h, than 10 cm^3 0.3 mol l^{-1} TCA was added, clarified solutions was used as blank). Proteolytic enzyme activity was expressed in HbU.

$$1 \text{ HbU} = \frac{\text{Absorbance of the sample at 660 nm} \times k \times 16}{\text{solids content of sample (g)}}$$

1.5. Determination of nucleic acid content

Twenty-five mg of dried yeast were heated for 20 min at 100 °C with 50 cm^3 of 1 *N* perchloric acid. The optical density of the centrifuged supernatant was then measured in a spectrophotometer at 270 nm against 1 *N* perchloric acid. The nucleic acid content was estimated from a calibration curve according to MUAYAD (1983). The RNA content of yeast biomass was measured after 4 and 4.5 h fermentation time, respectively.

1.6. Determination of protein content

Determination was carried out in the Kjel-Foss automatic equipment. Dried yeast biomass of 0.5 g was used. The protein content was expressed as N×6.25 g in 100 g solids (MUAYAD, 1983). Protein content of yeast cells was determined at the end of the batch fermentation in column fermentors.

2. Results

2.1. The effect of date syrup concentration on yeast growth rate

In our experiments we used date syrup media at concentrations of 0.1, 0.3, 0.5, 0.8 and 1% supplemented with 0.5% yeast extract. As a control, synthetic medium + 0.5% glucose (1.1.2.) was used. As fermentation equipment, a column fermentor (1.3.3.) was used at a constant aeration level of 600 $dm^3\ h^{-1}$. Yeast inoculum was grown in shaken culture and the sterile medium was inoculated with an amount of cells to get a starting concentration of 0.2% yeast solids. Cell propagation was followed by measuring absorbance of fermentation broth every hour. Fermentation was stopped when no further increase in cell density could be detected. This was after 4.5 h for *S. cerevisiae* and after 4 h in case for *C. guilliermondii* and *R. glutinis*.

Figures 1a, 1b show the growth curve for the investigated yeast strains with different date syrup concentrations and synthetic media with 0.5% glucose, respectively. Growth rate increased for each strain by increasing the carbon source content from 0.1% to 1.0%. There is no significant difference between the effect of 0.5%, 0.8% and 1.0% date syrup concentration ($\alpha \leq 0.01$). Glucose medium at 0.5% resulted in a significantly lower growth rate than 0.5% date syrup medium in case of *R. glutinis* ($\alpha \leq 0.01$).

At each investigated date syrup concentration and also in the synthetic medium *C. guilliermondii* and *R. glutinis* showed a better growth expressed in $\frac{\Delta \mathrm{OD}}{\Delta \mathrm{time}}$ than *S. cerevisiae* (Fig. 1).

2.2. The effect of date syrup concentration on protein content of yeast biomass

Figure 2 shows the protein content of the investigated yeast strains propagated at different date syrup concentrations. The protein content of *S. cerevisiae* increased from 50 ± 0.28% to 54 ± 0.98% when the date syrup content was increased from 0.1 to 0.3%. Further date syrup addition had no significant effect ($\alpha \leq 0.01$) on protein concentration. *C. guilliermondii* protein content showed no significant change with increasing carbon source content, and with *R. glutinis* a gradual decrease of protein content could be observed.

If we calculated the whole assimilated protein from cell concentration and protein content, we can see that the amount of protein synthesized by the cells increases at higher date syrup concentrations (Table 4) for each strain. Total synthesized protein (protein % × yeast conc.) is, at each date syrup concentration higher for *S. cerevisiae* than for *C. guilliermondii*. Protein amounts produced at 0.5% glucose concentration were slightly lower than those determined at 0.5% date syrup medium (Table 4).

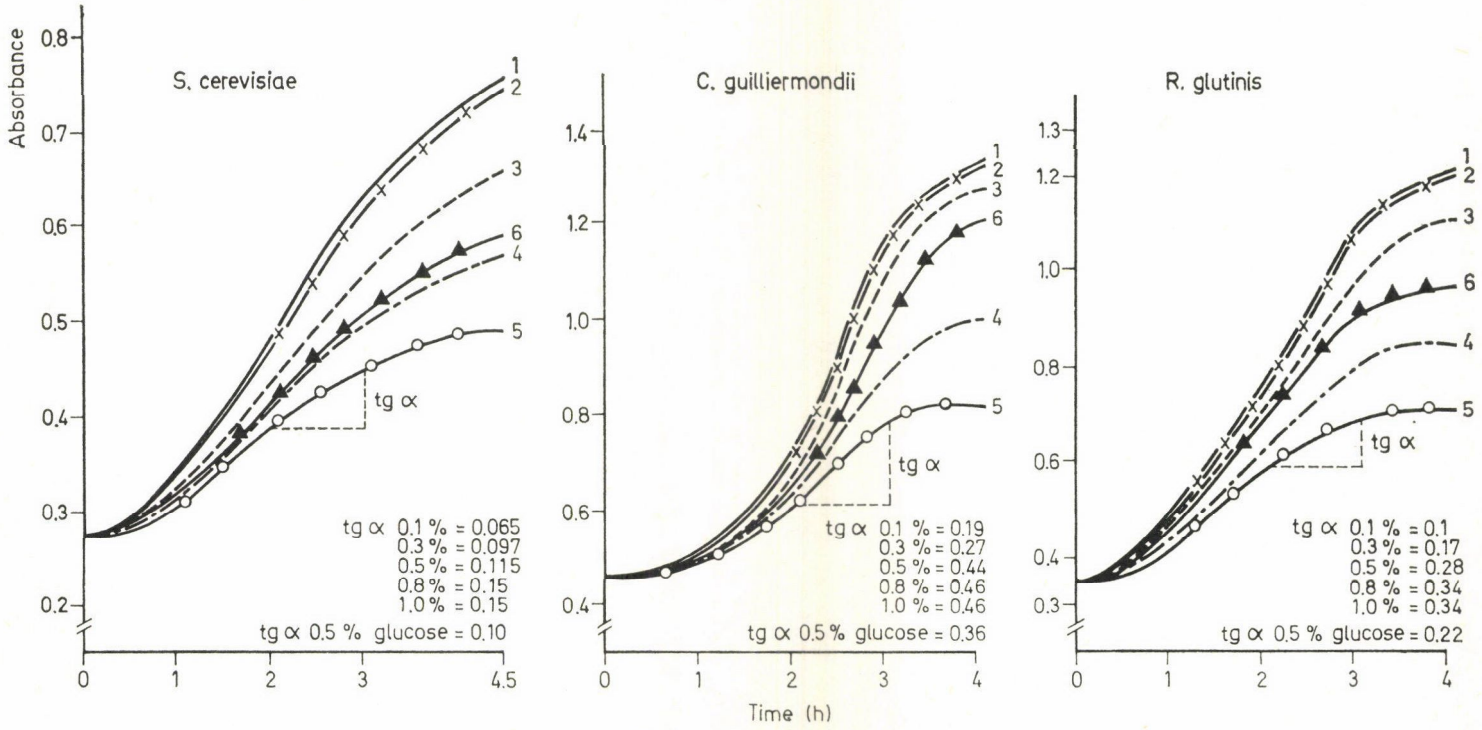


Fig. 1a. Growth rate of yeast (g per 100 cm³). Concentration of date syrup 1 : 1.0%; 2 : 0.8%; 3: 0.5%; 4: 0.3; 5: 0.1%; 6: synthetic media + 0.5% glucose

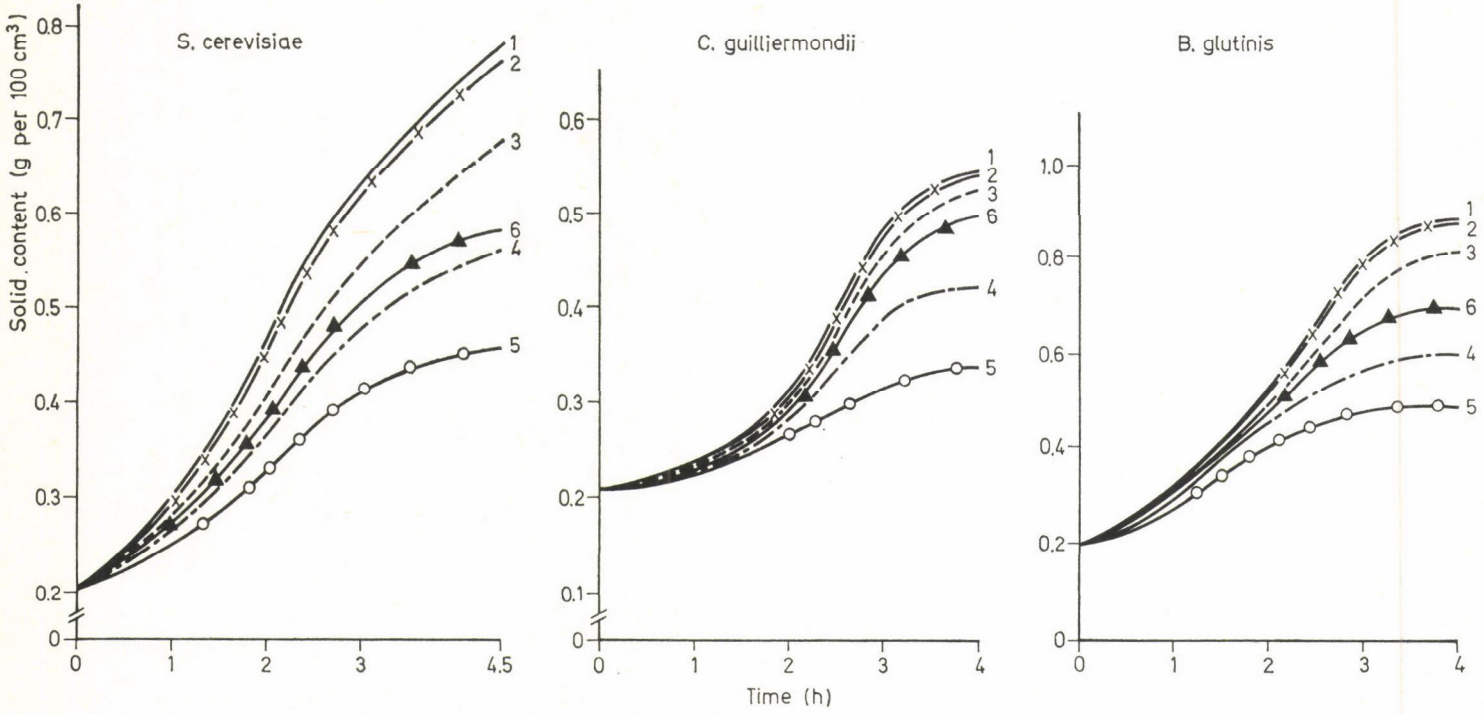


Fig. 1b. Growth rate of yeast (g per 100 cm³). Concentration of date syrup 1: 1.0%; 2: 0.8%; 3: 0.5%; 4: 0.3%; 5: 0.1%; 6: synthetic media + 0.5% glucose

Table 1

The effect of date syrup concentration on growth rate of S. cerevisiae

(Significance test)

Concentration of date syrup (%)	Concentration of date syrup (%)					Concentration of glucose (%)
	0.1	0.3	0.5	0.8	1.0	0.5
0.1		Ø	Ø	**	**	Ø
0.3			Ø	Ø	Ø	Ø
0.5				Ø	Ø	Ø
0.8					Ø	Ø
1.0						Ø

Ø: no significant difference
**: highly significant at $P \geq 99\%$ probability level

Table 2

The effect of date syrup concentration on growth rate of R. glutinis

(Significance test)

Concentration of date syrup (%)	Concentration of date syrup (%)					Concentration of glucose (%)
	0.1	0.3	0.5	0.8	1.0	0.5
0.1		Ø	**	**	**	*
0.3			*	*	*	*
0.5				Ø	Ø	Ø
0.8					Ø	Ø
1.0						Ø

Ø: no significant difference
*: significant at $P \geq 95\%$ probability level
**: highly significant at $P \geq 99\%$ probability level

Especially surprising are the results if we compare protein contents of the different yeast strains, as at each date syrup concentration (except 0.1%) the harvested *S. cerevisiae* had a significantly higher protein content than the other two investigated strains, which are known as high protein containing yeasts.

Thus the differences in protein contents are caused by the highly different growth rates which resulted in yeast cells of different growth stages after 4 and 4.5 of fermentation time, resp., and also in different degree of depletion of the C and N sources in the medium.

Table 3

The effect of date syrup concentration on growth rate of C. guilliermondii

(Significance test)

Concentration of date syrup (%)	Concentration of date syrup (%)					Concentration of glucose (%)
	0.1	0.3	0.5	0.8	1.0	0.5
0.1		Ø	**	**	**	*
0.3			*	**	**	Ø
0.5				Ø	Ø	Ø
0.8					Ø	*
1.0						*

Ø: no significant difference
*: significant at $P \geq 95\%$ probability level
**: highly significant at $P \geq 99\%$ probability level

2.3. *The effect of date syrup concentration on the RNS content of yeast biomass*

Figure 3 shows RNA concentrations at different date syrup concentrations for *S. cerevisiae*, *C. guilliermondii* and *R. glutinis*.

At the lower carbon source concentrations (0.1 and 0.3%) *S. cerevisiae* biomass had the lowest RNA content.

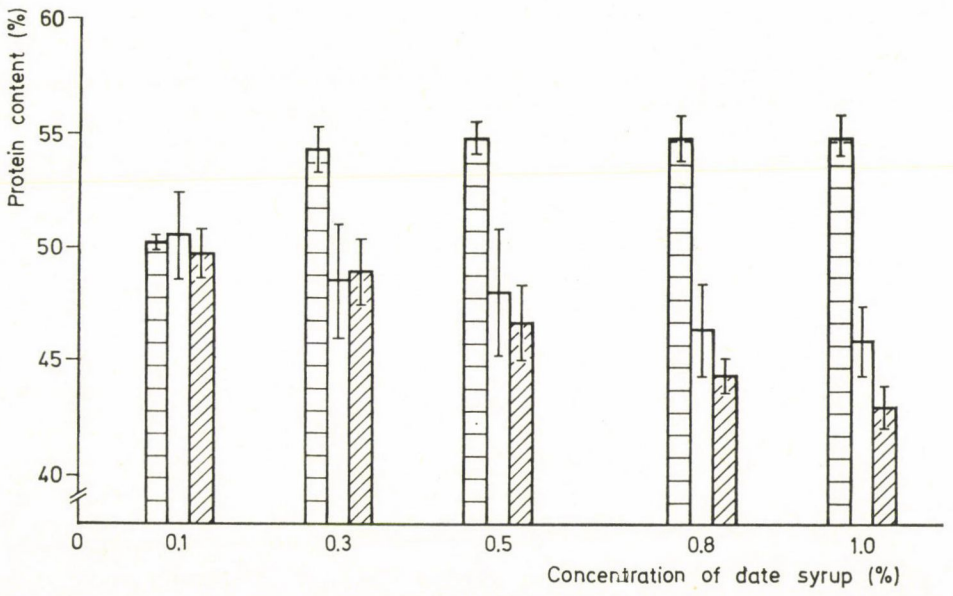


Fig. 2. The effect of date syrup concentration on protein content of yeast ▤: *S. cerevisiae*; ▯: *C. guilliermondii*; ▨: *R. glutinis*

Table 4

The effect of sugar concentration on protein production at the end of fermentation

Concentration of sugar (%)	Protein production [protein (%) × yeast (dry weight) concentration]					
	S. cerevisiae		*C. guilliermondii*		*R. glutinis*	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Date syrup						
0.1	19.57	0.97	16.17	0.8	23.88	1.19
0.3	26.06	1.3	18.93	0.94	29.8	1.49
0.5	30.08	1.5	22.8	1.14	38.34	1.91
0.8	35.89	1.79	23.66	1.18	39.51	1.97
1.0	36.99	1.84	23.6	1.18	38.23	1.91
Glucose						
0.5	27.8	1.39	20.42	1.02	33.90	1.69

Table 5

The effect of date syrup concentration on protein content of S. cerevisiae

(Significance test)

Concentration of date syrup %	Concentration of date syrup (%)				
	0.1	0.3	0.5	0.8	1.0
0.1		*	*	*	*
0.3			Ø	Ø	Ø
0.5				Ø	Ø
0.8					Ø
1.0					

Ø: no significant difference
*: significant at P $\geq$ 95% probability level

This is in agreement with the much lower growth rate of *S. cerevisiae*. At 0.5% date syrup content, RNA values show significant difference between yeast strains (Fig. 3). At 0.8% date syrup in Tables 7 and 7a there are not ignificantly different RNA values. At 1% date syrup concentration *S. cerevisiae* had the highest RNA content.

For *S. cerevisiae*, the increase in date syrup caused a gradual increase in RNA concentration in parallel to the change in growth rate.

The RNA concentration of *C. guilliermondii* showed no significant change with increasing date syrup content. For *R. glutinis*, the maximum RNA

Table 6

The effect of date syrup concentration on protein content of R. glutinis
(Significance test)

Concentration of date syrup (%)	Concentration of date syrup (%)				
	0.1	0.3	0.5	0.8	1.0
0.1		Ø	*	**	**
0.3			Ø	**	**
0.5				*	**
0.8					Ø
1.0					

Ø: no significant difference
*: significant at $P \geq 95\%$ probability level
**: highly significant difference at $P \geq 99\%$ probability level

content was at 0.3%, and a further increase in date syrup content brought about a decrease in RNA concentration (Table 8).

As the growth rates of each strain are significantly different in date syrup media of different concentrations the differences in RNA contents could be explained by the different growth stages of the yeast cells i.e. at higher carbon source concentrations the culture reaches the steady state phase earlier.

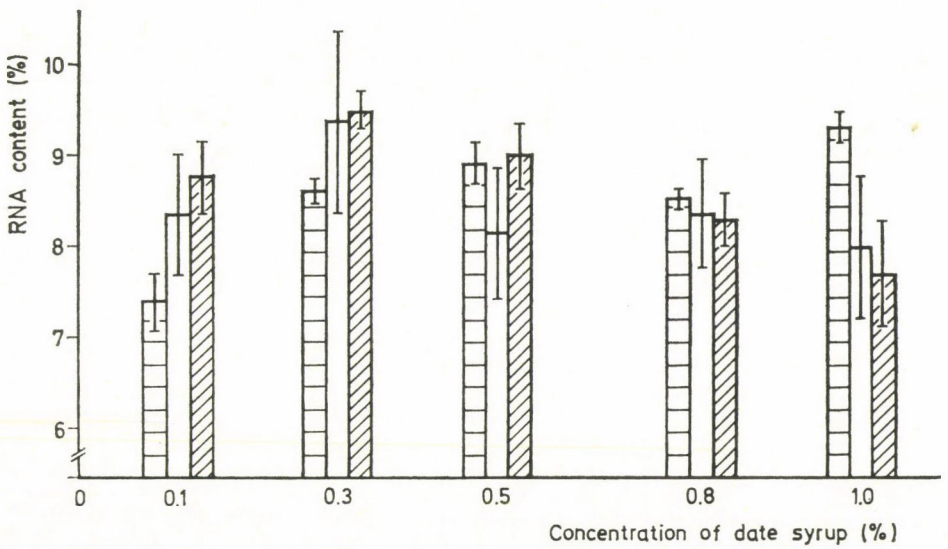


Fig. 3. The effect of date syrup concentration on RNA content of yeasts ▤: *S. cerevisiae*; ▭: *C. guilliermondii*; ▨: *R. glutinis*

Table 7

The effect of date syrup concentration on RNA content of S. cerevisiae
(Significance test)

Concentration of date syrup (%)	Concentration of date syrup (%)				
	0.1	0.3	0.5	0.8	1.0
0.1		**	**	**	**
0.3			*	Ø	**
0.5				*	**
0.8					**
1.0					

Ø: no significant difference
*: significant at $P \geq 95\%$ probability level
**: highly significant at $P \geq 99\%$ probability level

Table 7a

RNA content of different yeast strains at 0.5% date syrup
(Significance test)

	R. glutinis	*C. guilliermondii*
S. cerevisiae	Ø	*
C. guilliermondii	**	

Ø: no significant difference
*: significant at $P \geq 95\%$ probability level
**: highly significant at $P \geq 99\%$ probability level

Table 8

The effect of date syrup concentration on RNA content of R. glutinis
(Significance test)

Concentration of date syrup (%)	Concentration of date syrup (%)				
	0.1	0.3	0.5	0.8	1.0
0.1		*	Ø	Ø	**
0.3			Ø	**	**
0.5				*	**
0.8					*
1.0					

Ø: no significant difference
*: significant at $P \geq 95\%$ probability level
**: highly significant at $P \geq 99\%$ probability level

2.4. The effect of date syrup concentration on the proteinase activity of yeasts

The proteolytic enzyme activity of the investigated yeast strains was measured after the end of the 4 and 4.5 h batch fermentations, respectively. At each date syrup content, *S. cerevisiae* showed the highest proteolytic activity with a maximum value at 0.5% date syrup concentration (Fig. 4).

C. guilliermondii yeast had a minimum protease activity at 0.1% date syrup concentration. The enzyme activity significantly changed in the ranges of 0.3–1.0% date syrup concentration. Maximum value was at 0.5% carbon source content.

Table 9

The effect of date syrup concentration on proteinase activity of S. cerevisiae

(Significance test)

Concentration of date syrup (%)	Concentration of date syrup (%)				
	0.1	0.3	0.5	0.8	1.0
0.1		*	**	Ø	Ø
0.3			**	Ø	Ø
0.5				**	**
0.8					Ø
1.0					

Ø: no significant difference
*: significant at P ≥ 95% probability level
**: highly significant at P ≥ 99% probability level

Table 10

The effect of date syrup concentration on proteinase activity of C. guilliermondii

(Significance test)

Concentration of date syrup (%)	Concentration of date syrup (%)				
	0.1	0.3	0.5	0.8	1.0
0.1		**	**	**	**
0.3			**	Ø	**
0.5				**	**
0.8					**
1.0					

Ø: no significant difference
*: significant at P ≥ 95% probability level
**: highly significant at P ≥ 99% probability level

Table 11

The effect of date syrup concentration on proteinase activity of R. glutinis
(Significance test)

Concentration of date syrup (%)	Concentration of date syrup (%)				
	0.1	0.3	0.5	0.8	1.0
0.1		**	**	**	**
0.3			**	*	Ø
0.5				Ø	**
0.8					**
1.0					

Ø: no significant difference
*: significant at $P \geq 95\%$ probability level
**: highly significant at $P \geq 99\%$ probability level

The protease activity of *R. glutinis* decreased in the range of 0.1–0.5% date syrup content from 24.8×10^{-3} to 13.5×10^{-3} HbU g^{-1}.

In the concentration range of 0.5–1.0% date syrup content, a significant increase in protease activity could be detected.

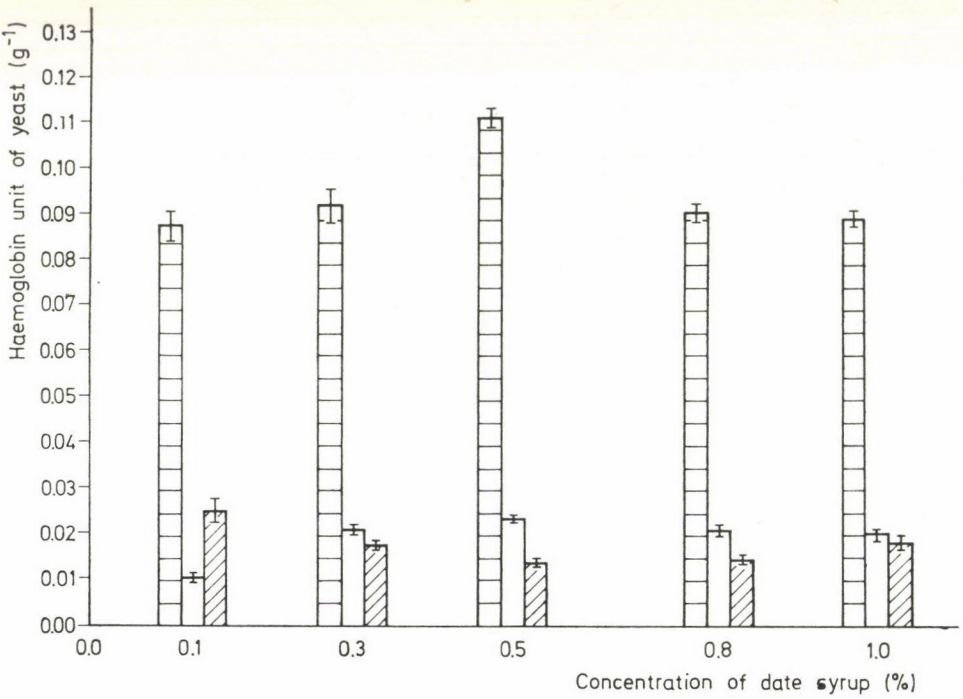


Fig. 4. The effect of date syrup concentration on proteinase activity of yeasts (aeration 600 l h^{-1}) ▤: *S. cerevisiae*; ▭: *C. guilliermondii*; ▨: *R. glutinis*

The proteolytic enzyme activity of *C. guilliermondii* showed a similar variation with date syrup concentration as that of *S. cerevisiae*, but the absolute values were significantly lower in each case.

3. Conclusions

Our experiments showed that date syrup is a good substrate for yeast strains of different genera. In batch fermentation, a date syrup concentration of 0.8–1% gives best growth rates for *S. cerevisiae*, *C. guilliermondii* and *R. glutinis* as well.

As regards biomass production *C. guilliermondii* and *R. glutinis* gave much better results in cell concentration. Total synthesized protein mass values were best for *S. cerevisiae* followed by *C. guilliermondii*. However, the faster growing yeast strains should be harvested after a shorter fermentation time to get higher protein concentration on solids. Without further substrate addition the protein content of *C. guilliermondii* and *R. glutinis* was 50.5% and 49.7%, resp., and for *S. cerevisiae* 50.2%, at 0.1% date syrup concentration. In the case of sufficient C-source concentration (0.5%), protein concentrations are 45.9%, 48.9% and 54%, respectively. It is also possible that for the fast growing yeast the N-source content of date syrup should be supplemented.

RNA content of the investigated yeast strains was differently influenced by C-source content. For *S. cerevisiae* the increase in date syrup content caused gradual increase in RNA concentration parallel to the change in growth rate. For the faster growing yeast strains the RNA concentration showed no significant change with increasing date syrup content *(C. guilliermondii)* or decreased at C-source concentration higher than 0.3% *(R. glutinis)*. These phenomena could be explained by the different growth phases of the investigated biomasses caused by the very different growth rates. At higher carbon source concentrations the culture reaches the steady state phase earlier. The N-source content of the date syrup medium could be the limiting factor in case of the fast growing yeast.

While the proteolytic activities of *S. cerevisiae* and *C. guilliermondii* changed similarly with increasing C-source concentration, the enzyme activity of *S. cerevisiae* was significantly higher. Protease activity of *R. glutinis* showed minimum value at 0.5% date syrup concentration and highest value at 0.1%. The proteolytic activity of *R. glutinis* was lowest at each C-source concentration, except 0.1%.

This difference in protease changes with date syrup concentration could be caused by the difference in glucose metabolism of *R. glutinis* and *S. cerevisiae* and *C. guilliermondii*, respectively.

Literature

ACHSTETTER, T., EHMANN, C. & WOLF, D. H. (1982): Aminopeptidase Co, a new yeast peptidase. *Biochem. Biophys. Res. Commun., 109*, 341–347.

ANSON, M. L. (1939): The estimation of pepsin, trypsin, papain and cathepsin with haemoglobin. *J. Gen. Physiol. 22*, 79–89.

AL-RAWI, N., MARKAKIS & BAUER, D. H. (1967): Amino acid composition of Iraqi dates. *J. Sci. Fd Agric., 18*, 1–2.

HEINRITZ, B., ROGGE, G., STICHEL, E. & BLEY, T. H. (1983): Use of biorhythmic processes for increasing the efficiency of carbon-compound conversion by microorganisms. *Acta Biotechn., 3*, 125–131.

HINZE, H., BETZ, H., SAHEKI, T. & HOLZER, H. (1975): Formation of complex between yeast proteinases A and B. *Hoppe-Seyler's Z. physiol. Chem., 356*, 1259–1264.

HOLZER, H. (1975): Regulatory role of proteinases and proteinase inhibitors in yeast. — in: KELETI, T. (Ed.) *Proc. 9th FEBS Meeting (Mechanism of action and regulation of enzymes.)* Akadémiai Kiadó, Budapest, Vol. 32, pp. 181–193.

HOLZER, H. (1977): Characteristics and functions of proteinase and proteinase inhibitors in yeast. — in: SHALTED, S. (Ed.) *Metabolic interconversion of enzymes.* Springer Verlag, Berlin, pp. 168–173.

KAMEL, B. S. (1979): Utilization of date carbohydrate as substrate in microbial fermentation. *Process Biochem., 5*, 14–18.

LENNEY, J. F. (1955): A study of two yeast proteinases. *American Chemical Society, Cleveland.* (Proc. Conf., 1951), pp. 919–930.

MOHAMED, A. M. & AHMED, A. A. (1981): Libyan date syrup (Rub Al-Tamr). *J. Fd Sci., 46*, 1162–1166.

MUAYAD, A. R. & KOJI (1983): Production of methionine rich yeast biomass for human consumption. PhD Thesis, Budapest.

SACHDE, A. G., AL-KIASY, A. & NORRIS, R. A. (1981): A study of the possibility of producing quality wine from some commercial varieties of Iraqi dates. *Mesopotamia J. Agr., 16*, 93–106.

SAKURADA, T. (1966): Nitrogenous compounds of *S. cerevisiae* grown on different carbon sources. *Keio J. Med., 15*, 45–50.

SELEM, S. A. & HEGAZI, S. M. (1971): Chemical composition of the Egyptian dry date. *J. Sci. Fd Agric., 22*, 632–633.

WILLIAM, M. F. (1983): *Microbial enzymes and biotechnology.* Applied Science Publishers, London and New York, pp. 250–305.

WOLF, D. H. (1982): Proteinase action in vitro versus proteinase function in vivo mutants on intercellular proteolysis in yeast. *Trends Biochem. Sci., 7*, 35–37.

Acta Alimentaria, Vol. 18 (2), pp. 193—201 (1989)

NUTRITIONAL EVALUATION OF *ACACIA ARABICA SEED*

C. R. MAITY[a] and B. MANDAL[b]

[a]Department of Biochemistry, Burdwan Medical College, Burdwan-713104, West Bengal. India
[b]Department of Chemistry, B. N. Mahavidyalaya, Itachuna P. O. Hooghly-712147, West Bengal. India

(Received: 4 February 1988; accepted: 12 August 1988)

Deoiled seed cake of *Acacia arabica* was analysed for its nutrient composition and amino acid profile. The deoiled seed cake contained 21.9% protein and balanced amino acids but also contained antinutritional factors, tannins (4.2%) and saponins (2.4%). Detoxification of the seed meal was done by treatment with lime and subsequent autoclaving. The nutrient and amino acid composition of the detoxified seed meal of *A. arabica* (PAM) was almost similar to that of unprocessed seed meal except for the antinutritional factors. The PAM was nutritionally evaluated by rat bioassay procedure in a comparative study with casein as standard. Nutritional indices, biochemical parameters and histopathological findings indicated the possibility of using the PAM as supplementary feed for livestock animals.

Keywords: *Acacia arabica*, unconventional legume seed, alkali detoxification, nutritional evaluation

Acacia arabica is a moderate sized ever green spiny tree, naturalised all over India and is capable of growing even under inhospitable and acute adverse ecological conditions. A mature tree can produce about 150–200 kg of seed-bearing green pods. The pods are generally 10–12 cm in length and each consists of 8–10 seeds. The deseeded pods containing 18–27% of tannins are used in village tanneries. The seeds at present go as a waste material. Local farmers use the green seed-bearing pods in the feedstuffs for cattle. The pods are also well accepted by the cattle. This fact induced the authors to study this largely available wild legumes. During studies, we observed that urine voided by the ruminants on green *A. arabica* pod ration showed flaky sediment which on treatment with dilute HCl hydrolysed to liberate glucose. It was also found by the authors that inclusion of 5–7 kg green pods per day in the diet of cows for a week resulted suffering from primary indigestion characterised by less feed intake, suspension of rumination and 2–5% fall in milk yield. The most plausible explanation for this phenomenon is the presence of tannins in the pods. Yet it may be attempted to assess the possibilities of including the green seed-bearing pods in the ration of ruminants because of large availability, good palatability and as they are the source of low-cost supplementary protein. Though this unconventional food source

Akadémiai Kiadó, Budapest

needs to be detoxified prior to its use in animal feedstuffs. Such studies are in progress. As a part of this study, chemical composition and food value of the deoiled seed cake of *A. arabica* is reported here.

1. Materials and methods

The mature *Acacia arabica* (fam: Leguminosae) pods were collected from the local forests of Hooghly district (WB), India and seeds were removed from air dried pods.

1.1. Physical characteristics of the seed

The physical dimensions (length, breadth and thickness) of 25 seeds of varying sizes were measured by means of a vernier caliper. The volume of 100 seeds determined by dropping the seeds into a known volume of water in a measuring cylinder and noting the increase in volume.

1.2. Analysis of the seed cake

Dried seeds were ground and defatted by solvent (n-hexane) extraction method in a Soxhlet distillation apparatus for 72 h and air dried to remove the traces of solvent. Defatted *A. arabica* seed meal (CAM) was analysed for moisture, crude protein, carbohydrates, crude fibre and ash following the methods as described by RACHURAMULU and co-workers (1983). Tannin content was estimated following the gravimetric method based on the absorption of tannins by hide power as described by ATTAL and co-workers (1978). Saponin content was determined by the method of VAN ATTA and co-workers (1961). Amino acid analysis was done by column chromatography as described earlier by us (MANDAL et al., 1982; 1984a).

1.3. Processing of the seed meal

Deoiled seed cake was processed following the same method as described earlier for *A. auriculaeformis* seed meal (MANDAL et al., 1987). In this simple method, one kg of the meal (CAM) was mixed with 50 g of lime and 250 cm^3 of water and subsequently autoclaved at 110 °C for 10 min. The dried and processed seed meal (PAM) was stored in cold until use.

1.4. Analysis of the processed seed meal (PAM)

PAM was analysed for moisture, crude protein, carbohydrates, crude fibre, ash, tannins, saponins and amino acid composition.

1.5. Nutritional evaluation of PAM

PAM was nutritionally evaluated by rat bioassay procedure and comparison with casein was done.

1.5.1. Animals and diets. Growing male albino rats of local strain (inbred in our laboratory) weighing about 45–50 g of 25–28 days were used in this study. The animals were individually caged and maintained under controlled temperature (20–24 °C) and humidity (55%) conditions. Twenty four animals were divided into two groups, twelve in each and equalized with respect to body weight. One group of animals were fed on casein-diet and another group was assigned to diet containing PAM. Composition of the casein-diet and PAM-diet is shown in Table 1. PAM was incorporated at the level of 35% as the total nitrogen of the PAM-diet became 16 g per kg, i.e., protein at the level of 10%; casein-diet also contained protein at about 10% level. The experimental animals received their respective diet and water ad libitum for 28 days. Food intake was recorded daily and body weight twice weekly.

1.5.2. Nutritional indices. Protein efficiency ratio (PER) was calculated from values for body weight gain and crude protein (CP) (N content of the test diet $\times$ 6.25) intake over a 28 day period. PER was calculated as body weight gain per unit CP intake.

1.5.3. Biochemical estimations. At the end of 28 days, animals were sacrificed and blood was collected for haematological and biochemical estimations. Blood haemoglobin (Hb) was measured using haemoglobinometer

Table 1

Composition of the experimental diet

Ingredients	Casein-diet (g kg^{-1})	PAM-diet (g kg^{-1})
Starch	535	300
Sucrose	200	200
Cellulose powder	50	50
Groundnut oil	50	50
Mineral salts[a]	40	40
Vitamin mixture[a]	10	10
Casein	115	—
PAM	—	350
Total	1000	1000
Total N (by analysis)	16.7	16.0

[a] According to MANDAL et al. (1982)
PAM: Processed *Acacia arabica* seed meal
N: nitrogen

(Coulter Electronics, Hialeah, Florida, USA). Blood sugar was estimated following the method of SOMOGYI (1945). Estimation of serum total protein was done by folin-phenol method (LOWRY et al., 1951). Total lipid materials

Table 2

Physical characteristics of seed and nutrient and amino acid composition of Acacia arabica *seed meal*

Physical characteristics of seed:		
Weight of 100 seeds (g)	15.1	
Volume per 100 seeds (cm^3)	11.6	
Length (mm)	8—10	
Breadth (mm)	6—8	
Width (mm)	2—4	
Oil in seed (%)[a]	5.2	
Nutrient composition:	CAM	PAM
Total carbohydrates (%)	45.8	44.6
Total N (%)	3.51	3.45
Total moisture (%)	10.4	7.6
Total ash (%)	4.2	4.8
Crude fibre (%)	12.6	12.8
Crude fat (%)	2.6	2.4
Tannins (%)	4.2	—
Saponins (%)	2.4	0.8
Amino acid profile (g per 16 g N)[b]:		
Glycine	6.1	5.4
Alanine	5.1	5.2
Threonine	13.9	12.6
Serine	6.9	6.2
Valine	4.2	4.1
Leucine	3.9	3.6
Isoleucine	4.1	4.3
Proline	4.0	4.6
Phenylalanine	4.4	4.1
Methionine	0.9	0.4
Tyrosine	4.6	3.2
Histidine	3.0	2.6
Arginine	8.4	7.8
Lysine	5.6	5.4
Aspartic acid	6.1	5.2
Glutamic acid	10.8	10.0

Data are mean values of three determinations
Tryptophan was not determined
[a] On dry basis
[b] Values not corrected for any loss due to acid hydrolysis
N: nitrogen
CAM: unprocessed *Acacia arabica* seed meal
PAM: processed *Acacia arabica* seed meal

of serum was extracted and washed according to the method of FOLCH and co-workers (1957). Total serum cholesterol was determined by the method of SPERRY and WEBB (1950).

1.5.4. Histopathological examination. Some key organs especially liver, kidney and testis were collected immediately after sacrifice, weighed and processed for histopathological findings. Paraffinised sections (7 μ) of these organs were stained with haematoxylin-eosin and examined microscopically.

2. Results

2.1. Composition of the seed meal

Acacia arabica seeds are dark brown, shiny and flat hard seeds with thick strong shells. Physical characteristics of the seed and analytical composition including amino acid composition of the deoiled seed cake before and after detoxification are shown in Table 2. The seed length varied between 8 and 10 mm while breadth varied from 6 to 8 mm.

Oil content of the seed is 5.2%. Analytical composition of the deoiled seed meal showed the presence of crude protein at a level of 21.9% (N×6.25) and carbohydrates at a level of 45.8%. The defatted seed cake also contains antinutritional factors, tannins (4.2%) and saponins (2.4%). Detoxification by lime treatment caused the reduction of saponins and complete inactivation of the tannins. The nutrient composition of the processed seed meal (PAM) is almost similar to that of unprocessed meal (CAM) except for the antinutri-

Table 3

Recommended essential amino acid (g per 16 g N) pattern of FAO/WHO (1973) for laboratory animals and essential amino acid contents (g per 16 g N) of PAM

	FAO/WHO (1973)	PAM
Phenylalanine + Tyrosine	6.0[a]	7.5[a]
Isoleucine	4.0	4.3
Leucine	7.0	3.6
Lysine	5.5	5.4
Methionine + Cystine	3.5[a]	0.4[a]
Threonine	4.0	12.6
Valine	5.0	4.1
Arginine	—	7.8

[a]: Combined value for the two amino acids
—: actual data is not available
PAM: processed *Acacia arabica* seed meal

tional factors. The seed protein consists of 16 usually occurring amino acids and the detoxification process did not show any appreciable effect on the amino acid composition.

Recommended essential amino acid pattern of FAO/WHO (1973) for laboratory animals and essential amino acid contents of PAM are given in Table 3. In comparison with the FAO/WHO (1973) amino acid pattern, PAM contained insufficient methionine-cystine and essential amino acids, leucine and valine were also present at levels that were somewhat lower than those recommended by FAO/WHO (1973).

2.2. Nutritional performances of the PAM

Values for the nutritional indices and blood biochemical parameters of rats fed diets containing casein and PAM for 28 days are summarized in Table 4. Crude protein (CP) intake was about non-significantly 15% lower for the PAM-diet. Body weight gain for the rats fed casein-diet was significantly higher ($P \leq 0.001$) compared to those fed PAM-diet. Protein efficiency ratio (PER) of PAM-diet was significantly lower ($P \leq 0.02$) than that of casein-diet. The values for blood biochemical parameters of rats fed PAM-diet were normal and compared well to those fed casein-diet. Two dietary groups showed no significant variation with regard to biochemical parameters. No histopathological abnormalities were seen in the various organs of rats fed PAM-diet for 28 days.

Table 4

Nutritional indices and biochemical parameters of rats fed casein-diet and PAM-diet for 28 days

	Casein-diet		PAM-diet	
	$\bar{x}$	±s	$\bar{x}$	±s
Nutritional indices: CP intake (g) for 28 days	24.6	1.5	20.8	1.8
Body weight gain (g) for 28 days	68.1	2.1	38.4**	3.8
PER	2.7	0.21	1.84*	0.26
Biochemical parameters:				
Haemoglobin (g per 100 cm^3)	14.8	0.8	14.6	0.7
Blood sugar (mg per 100 cm^3)	86.2	8.2	78.8	7.6
Serum total protein (g per 100 cm^3)	5.2	0.8	5.2	0.6
Serum total lipids (mg per 100 cm^3)	268.5	9.8	256.9	8.7
Serum total cholesterol (mg per 100 cm^3)	66.3	6.4	60.4	5.9

$\bar{x}$: mean value from twelve rats; ± s: standard deviation
CP: crude protein (N × 6.25)
PER: protein efficiency ratio
* Significant at $P \leq 0.02$ probability level
** Significant at $P \leq 0.001$ probability level
For diets see Table 1

3. Conclusions

3.1. Analytical composition of the seed cake

Our results recorded the smaller size of the seed compared to the reported value of 13 mm in length (COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, 1948). Extraction of the seed yielded 5.2% oil compared to the reported values of 5.4% (ECKEY, 1954) and 5.6% (SITA DEVI et al., 1979). The deoiled seed cake (CAM) contains 21.9% CP and 45.8% carbohydrates, therefore, may be used as a good source of protein and carbohydrates. The seed protein consists of 16 amino acids of which 8 are essential. Among the essential amino acids, sulphur containing amino acids — methionine and cystine are the most limiting which is an usual characteristic of the wild legumes especially Acacia seeds (PANT et al., 1974a; SUNDAR RAO et al., 1983). CAM also contains antinutritional factors, tannins (4.2%) and saponins (2.4%). The feeding value of theseed meal will be greatly impaired, however, by the presence of these undesirable components, though high protein content and its amino acid pattern suggest that it might be a valuable supplement in animal feed. It was found that rats fed diet containing 35% CAM for 28 days showed some metabolic and histopathological abnormalities (data are not presented). Therefore, antinutritional factors should be either removed or detoxified. In the present study, a simple method of tannin inactivation has been devised which does not adversely affect the nutrient and amino acid composition of the seed meal. Reduction of assayable tannins from some unconventional plant seeds (MANDAL et al., 1987) and high-tannin sal seed (KURAR & GUPTA, 1982) after treatment with a variety of alkalies has been described. The active groups in tannin molecule are polyphenolic hydroxyl groups which form hydrogen bonds with free carbonyl groups of the protein molecule. The mechanism of alkali-detoxification of hydrolysable tannins is that it blocks polyphenolic and carboxyl groups by alkali salt formation, i.e.,

$$\text{Tannin} + \text{Ca(OH)}_2 = \text{Ca-tannate} + \text{H}_2\text{O}$$

For an easy assessment of detoxification, a simple chemical test was done (MANDAL et al., 1985) which differentiated between the processed and unprocessed meals. A hot extract of the seed meal was concentrated and treated with 10 cm^3 of egg white, the processed meal gave no precipitate while the unprocessed meal gave a thick precipitate. This revealed that processing reduced the tannins to a level which had no longer the capacity to complex protein. Mechanism for the reduction of saponin content by this alkali-detoxification is not clear.

3.2. Possibility of the processed meal (PAM) as animal feed

Results of nutritional evaluation by animal feeding experiment show that CP intake of rats fed PAM-diet is about 84.5% of that with casein-diet while body weight gain for rats fed PAM-diet is about 56.3% of that with casein-diet. The PER value for the PAM-diet was also significantly lower than that obtained for casein. These suggest that PAM is nutritionally inferior to casein. One of the factors of this inferior nutritive value of the PAM is probably due to lack of some essential amino acids, particularly sulphur--containing amino acids. In contrast, the PER of the PAM compared well with those of some standard edible vegetable proteins (GOPALAN et al., 1984) and some wild legume seed protein isolates (PANT et al., 1974b; MANDAL et al., 1984b; 1986).

Values for the haematological and biochemical estimations of the rats fed PAM-diet are within the normal biological limit and did not significantly differ from those for the animals fed casein-diet. Histopathological abnormalities are not found in various organs of the animals fed diet containing PAM. These observations suggest that antinutritional or toxic factors in unprocessed seed meal have been properly inactivated due to alkali detoxification. On the basis of this study, it can be concluded that the chemical and nutritional qualities of the processed seed meal of *A. arabica* appear to be satisfactory as a source of low-cost protein and carbohydrates in livestock diet.

*

Financial assistance from the Indian National Science Academy (INSA), New Delhi, India (No. BS(RP-397/2620) to Dr. B. MANDAL is gratefully acknowledged. The authors are also grateful to the Principal, Burdwan Medical College for providing laboratory facilities for the feeding experiments of this study.

Literature

ATTAL, C. K., SRIVASTAVA, J. B. & WALLI, B. K. (1978): Screenings of Indian plants for biological activity. – Part VIII. *Indian J. exp. Biol., 16*, 330–340.

COUNCIL OF SCIENTIFIC and INDUSTRIAL RESEARCH (1948): *Wealth of India — raw materials*. New Delhi, Vol. 1, pp. 5–8.

ECKEY, E. W. (1954): *Vegetable oils and fats*. Interscience Publ. Inc., New York, p. 474.

FOLCH, J. M., LESS, M. & STANELY, G. H. S. (1957): A simple method for the isolation and purification of total lipids from animal tissue. *J. biol. Chem., 226*, 497–509.

FAO/WHO (1973): *Technical report series No. 522*. World Health Organisation, Geneva, p. 58.

GOPALAN, C., RAMASASTRI, B. V. & BALASUBRAMANIAN, S. C. (1984): *Nutritive value of Indian foods*. National Institute of Nutrition (NIN), Hyderabad, India, pp. 3–11.

KURAR, C. K. & GUPTA, B. N. (1982): Sal *(Shorea robusta)* seed meal in animal ration. — A review. *Agric. Rev., 3*, 109–117.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951): Protein measurement with folin-phenol reagent. *J. biol. Chem., 193*, 265–275.

MANDAL, B., GHOSH MAJUMDAR, S. & MAITY, C. R. (1982): Nutritional evaluation of the protein isolate obtained from the seed of *Eucalyptus kirtoniana* in albino rats — A comparative study. *Proc. Indian natn. Sci. Acad., B48*, 596–602

MANDAL, B., GHOSH MAJUMDAR, S. & MAITY, C. R. (1984a): Nutritional evaluation of nontraditional seed proteins with or without amino acid supplementation in albino rats. *Proc. Indian natn. Sci. Acad., B50*, 48–56.

MANDAL, B., GHOSH MAJUMDAR, S. & MAITY, C. R. (1984b): Nutritional evaluation of seed meal and seed protein. *J. Fd Sci. Technol., 21*, 307–312.

MANDAL, B., GHOSH MAJUMDAR, S. & MAITY, C. R. (1985): Processing and utilisation of nontraditional seeds from forest plants. *Proc. ISHS Symp. Med. Arom. Spice Pl., Spl. issue*, 123–130.

MANDAL, B., GHOSH MAJUMDAR, S. & MAITY, C. R. (1986): Isolation and evaluation of protein from processed karanja *(Pongamia glabra)* seed meal. *Acta Alimentaria, 15*, 69–77.

MANDAL, B., GHOSH MAJUMDAR, S. & MAITY, C. R. (1987): Processing and toxicological evaluation of akashmoni and eucalyptus seed meals. *Hum. Nutr. Fd Sci. Nutr.* (London) 41F, 135–143.

PANT, R., RAJAGOPALAN NAIR, C., SINGH, K. S. & KOSHTI, G. S. (1974a): Amino acid composition of some wild legumes. *Curr. Sci., 43*, 235–239.

PANT, R., RAJAGOPALAN NAIR, C., SINGH, K. S. & KOSHTI, G. S. (1974b): Nutritional evaluation of some Indian noncultivated wild leguminous seed protein isolates. *Curr. Sci., 43*, 773–776.

RAGHURAMULU, N., MADHAVAN NAIR, K. & KALYANASUNDARAM, S. (1983): *A manual of laboratory techniques*. National Institute of Nutrition (NIN), Hyderabad, India, pp. 31–36.

SITA DEVI, K., PANTULU, A. J. & LAKSHMINARAYANA, G. (1979): Analysis of babul *(Acacia nilotica)* seeds and oil. *J. Oil Technol. Ass. India, 11*, 65–66.

SOMOGYI, M. (1945): Determination of blood sugar. *J. biol. Chem., 160*, 69–73.

SPERRY, W. M. & WEBB, M. (1950: A revision of Schonheimer-Sperry method for cholesterol determination. *J. biol. Chem., 187*, 96–106.

SUNDAR RAO, K., PANTULU, A. J. & LAKSHMINARAYANA, G. (1983): Analysis of *Calotropis gigantea, Acacia caesia* and *Abelmoschus ficulneus* seeds. *J. Am. Oil. Chem. Soc., 60*, 1259–1261.

VAN ATTA, G. R., GUGGOLZ, J. & THOMPSON, C. R. (1961): Determination of saponins in alfalfa. *J. agric. Fd Chem., 99*, 77–82.

Acta Alimentaria, Vol. 18 (2), pp. 203—209 (1989)

INSECT DAMAGE TO WHEAT GRAIN

K. LORENZ

Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, Colorado 80523. USA

(Received: 4 February 1988; accepted: 6 September 1988)

Cages with insects *(Calocoris norvegicus, Sidnia kinbergi, Nysius huttoni)* were placed over plots of wheat at the flowering stage to determine any damage to the grain caused by feeding of the insects on the developing kernels. The insects remained inside the cages until harvest. Kernel weight, nitrogen, ash and falling number were determined. Flours milled from the grains were baked into breads. All grain samples showed insect attack. Kernel weight decreased and ash and protein contents of winter wheat increased due to insects feeding on the grain. Only *Sidnia kinbergi* caused statistically significant differences, however. Falling number was not affected. There was no detrimental effect on breadbaking quality except for a reduction in bread volume which was statistically significant. The question which insect(s) causes damage or under what conditions a particular insect causes the detrimental effects on grain protein known as "bug-damage" in New Zealand remains unanswered.

Keywords: insect damage, wheat, baking quality

Grain damage caused by insects has been reported from Germany, Russia, Spain, Hungary, Czechoslovakia, Yugoslavia, Italy, Turkey, Iran, Iraq and New Zealand (HIBRAOUI, 1930; BERLINER, 1931a, b; KRANZ, 1935; KRETOVICH, 1944; MEREDITH, 1970).

The insects responsible for the problem have been identified as *Eurygaster integriceps* and *Aelia rostrata* in Europe and the Near East and tentatively as *Nysius huttoni*, *Hudsona anceps* and *Stenotus binotatus* in New Zealand (MEREDITH, 1970). They are all sucking insects, piercing the developing grain with stylets. During feeding they supposedly inject saliva which contains very potent enzyme(s) causing drastic changes in the grain or flour. In New Zealand even mild infestation was found to produce runny, sticky doughs which cannot be handled in commercial bread production. It required only 3 to 4 damaged kernels in 1000 sound kernels to have a significant effect (MEREDITH, 1970). Heavy infestation in Europe caused reduced crop yields, kernels and straw developed an objectionable odour, and the flour was bitter and often not fit for human consumption (BERLINER, 1931a).

The damage is quite evident to the protein as concluded in the "slimy-gluten" studies even before the causal agent was identified (BERLINER, 1931a, b; KRANZ, 1935; HOPF, 1938). The damage is confined to the grains that show bite marks, though after grinding or milling to flour these subsequently

Akadémiai Kiadó, Budapest

affect the remainder. It seemed that damage is caused by a hydrolytic enzyme splitting the protein chains to smaller units (KRETOVICH, 1944). Work at the New Zealand Wheat Research Institute during the 1960s caused some doubt about the mechanism of simple hydrolysis, however, and a mechanism via reduction of interchain linkages of the proteins was considered (MEREDITH, 1970). The work failed to find a soluble factor. The effect was clearly enzymic, which is in line with Berliner's discussion of an unusual kind of enzyme which appeared to have special properties and was not apparent in water extracts of damaged grain (BERLINER, 1931a, b). More work remains to be done to elucidate mechanisms of damage to the protein in both the European and New Zealand situation.

Damage to the starch as well as to the protein is possible. Although KRANZ (1935) stated that diastatic power was at normal level in insect-damaged grain, MOHS and KLEMT (1936) found increased diastatic activity. They suggested that the effect was due to reduction of the resistance of the starch. KRETOVICH and TOKAREWA (1943) also reported increased diastatic activity in insect-damaged wheat. HOPF (1938) published photographs of starch granules that he said were corroded by insect secretions.

All in all, it seems logical that amylolytic activity should be found in some, but certainly not in all instances of insect damage.

The damage caused by bugs to the New Zealand wheat crop is usually low, but it varies considerably from year to year. The worst incidence for many years was in 1970, with over 500 affected samples being received at the Wheat Research Institute. This is a startling contrast with 1964 to 1969 when there were respectively only 16, 62, 2, 16, 21, and 50 affected samples received (MEREDITH, 1970). In recent years the problem has resurfaced in Syria, Russia, Bulgaria and New Zealand (GOTSOVA & KONTEV, 1981; EL-HARAMEIN et al., 1984; MEREDITH & BEST, 1985).

It was the purpose of this study to identify the insect(s) responsible for the damage to wheat in New Zealand. This has not been done previously in a controlled experiment with specific insect species.

1. Materials and methods

1.1. Grain identification and treatment

Three plots (3×1 m) of winter Rongotea wheat and 2 plots of spring Rongotea wheat were grown at the Crop Research Division, DSIR, Lincoln, New Zealand. The plots received 2 nitrogen superphosphate applications at 60 kg ha^{-1}. At the flowering stage cages were placed over the plots (Fig. 1). Plots No. 1 of the winter and spring wheats were used as controls. Into the

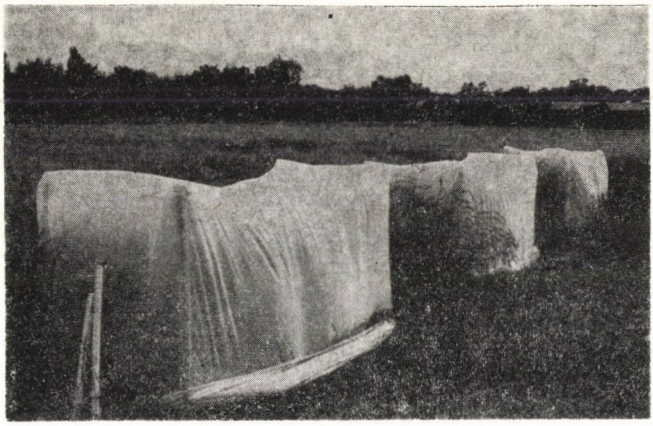


Fig. 1. Cages over experimental plots of wheat samples

remaining cages, 1500 insects per cage were introduced by the Entomology Division, DSIR, Lincoln; into cage No. 2 of winter wheat *Calocoris norvegicus*, into cage No. 3 *Sidnia kinbergi* and into cage No. 2 of the spring wheat *Nysius huttoni*. The insects remained inside the cages until harvest. *Nysius huttoni* is considered to be the insect responsible for damage to wheat grain.

Sidnia kinbergi and *Calocoris norvegicus* are often found in wheat fields in New Zealand. Their presence has thus far not been associated with any damage to wheat grains.

1.2. Scanning electron microscopy

Kernels of insect-damaged wheat showing bite marks were placed on specimen stubs, fastened and coated with gold-palladium and viewed with a Cambridge Stereo-scan 250 MK scanning electron microscope.

1.3. Proximate composition and kernel weight

Kernel weight, moisture, nitrogen, ash and falling number of each grain sample were determined by Approved Methods of the AACC and ISO (AACC, 1983; ISO, 1981). Statistical analysis was as outlined by SNEDECOR and COCHRAN (1980).

1.4. Milling and baking of samples

Grain from each of the 5 plots were tempered to 15% moisture and milled into flour on a Quadrumat Jr. mill (70 grit gauze). The flour was baked into pup loaves by the mechanical dough development process used in New

Zealand (CAWLEY, 1972). The formulation was as follows: flour 100%, salt 2%, fat 1.38%, sugar 0.75%, yeast 3%, ascorbic acid 100 ppm and potassium bromate 50 ppm. Doughs were developed by mixing to optimum in a Mitchell mixer (MITCHELL, 1971). After a rest period of 10 min each dough was molded and placed into baking pans for proofing. Proof time was 45 min at 35 °C and 90% R. H. and baking time 25 min at 221 °C. The loaves were removed from the pans and cooled on the bench. They were scored the following day by measuring volume objectively by rapeseed displacement and assessing other external and internal bread characteristics subjectively. To score the breads, a maximum number of points was given to each bread characteristic: crust colour, 7; symmetry, 7; break and shred, 6; crumb colour, 10; volume, 15; flavour, 15; grain, 20; and texture, 20.

2. Results and discussion

2.1. Grain appearance, kernel weights and proximate composition

Visual and microscopic examination of each grain sample, which had been in contact with insects, showed bite marks. A scanning electron micrograph of a bite mark on a kernel of wheat is shown in Fig. 2.

Kernel weights of winter Rongotea wheat decreased when insects *(Calocoris norvegicus* and *Sidnia kinbergi)* were introduced into the cages (Table 1). Only *Sidnia kinbergi* caused a statistical significant difference, however. The grain kernels were slightly shrivelled compared to the control sample. Ash and protein content increased with decreased kernel weight indicating an effect on starch deposition during grain development. Again,

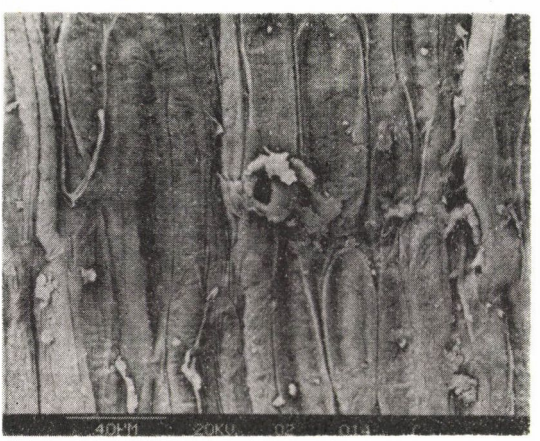


Fig. 2. Insect bite marks on kernel of wheat

Table 1

Proximate composition of insect-damaged wheat

Sample/Insect	1000 Kernel weight (g)	Ash (%)	Protein (%)	Falling number
Winter Rongotea-control	42.3a	1.56a	13.3a	379a
–*Calocoris norvegicus*	40.6a	1.58a	13.9b	379a
–*Sidnia kinbergi*	37.8b	1.75b	14.3b	444b
Spring Rongotea-control	37.0b	—	12.4c	371a
–*Nysius huttoni*	38.1b	—	12.6c	383a

Protein content: calculated on 14% moisture basis, and N × 5.7
Ash content: on 14% moisture bases
Different letters within a column indicate a significant difference at 0.05 level of significance

Table 2

Baking data
(Data are the averages of duplicate bakes)

Sample/Insect	Insect-damaged wheat				
	Flour yield (%)	Spec. vol. ($cm^3\ g^{-1}$)	Grain (20 pts max.)	Texture (20 pts max.)	Total score (100 pts max.)
Winter Rongotea--control	55.0a	6.00a	13a	17a	87a
–*Calocoris norvegicus*	53.6a	5.41b	16b	19b	90a
–*Sidnia kinbergi*	54.3a	5.36b	14a	18a	88a
Spring Rongotea--control	61.4b	4.72c	16b	17a	84b
–*Nysius huttoni*	57.9c	4.13d	16b	18a	83b

Different letters within a column indicate a significant difference at 0.05 level of significance

only *Sidnia kinbergi* caused a statistical significant difference in both ash and protein. Feeding of the insects on grain kernels did not result in decreased falling number values, which would be indicative of higher amylase activity.

The spring Rongotea wheat samples were lower in kernel weight compared with the winter wheat samples. There were no statistically significant differences in kernel weight, protein content and falling number due to insects in comparison with the control sample, which was surprising since *Nysius huttoni* is the insect which in the literature has been associated with "bug--damaged" wheat.

2.2. Milling and baking data

Quadrumat Jr. flour yields of winter Rongotea wheats were slightly lower due to the presence of insects *(Calocoris norvegicus* and *Sidnia kinbergi)* during grain development (Table 2). The differences were not statistically significant, however. Doughs mixed from the flours were dry and smooth and easy to handle. The volume of breads baked with the flours was significantly lower than that of the control. Other external (crust colour, overall appearance, crust character) and internal (grain, texture, crumb colour, flavour) characteristics of the breads, however, were as good or even better than those of the control bread. Piercing of the kernels did not affect baking quality.

Spring Rongotea wheat, which was exposed to *Nysius buttoni*, produced a significantly lower Quadrumat Jr. flour yield than the control sample. The flour, however, produced a dough with good handling characteristics. The bread was slightly lower in volume but equal in other external and internal characteristics compared to the control.

3. Conclusions

The 1500 insects per cage of *Calocoris norvegicus*, *Sidnia kinbergi* and *Nysius huttoni*, respectively, which had been in contact with the wheat from flowering until full grain maturity under controlled conditions, caused minor effects on kernel weight and grain composition but did not produce any detrimental effects on bread baking quality except a reduction in volume. This was surprising. Attack on wheat by *Nysius huttoni* has in the past been associated with runny, sticky doughs which produce bread of low volume. There were definitely more than 3 to 4 damaged kernels in 1000 sound kernels, required to have significant detrimental effect on baking characteristics (MEREDITH, 1970). The "bug-damaged" wheat in this study were certainly not "bug-damaged" as seen with some grain samples in the past in New Zealand and the question remains which insect(s) causes the damage or under what conditions does a particular insect cause the detrimental effects on grain protein known as "bug-damage".

*

Thanks go to Dr. D. WRIGHT, Crop Research Div. and Dr. J. FARELL and Mr. M. STUFKENS, Entomology Div., DSIR, Lincoln for the grain samples.

The financial support for this project (NRAC Fellowship) is gratefully acknowledged.

Literature

AACC (1983): Approved methods No. 46–12; 08–01 and 56–81B. American Association of Cereal Chemists, St. Paul, Minnesota.

BERLINER, E. (1931a): Der Leimkleber ein Gefahr für die Müller? *Mühlenlaboratorium, 1,* (1), 1.

BERLINER, E. (1931b): Leimkleber Weizen ist Wanzenweizen. *Mühlenlaboratorium, 1,* (4), 25–26.

CAWLEY, R. W. (1972): Mechanical dough development in New Zealand. *N. Z. Wheat Rev., 13,* 25.

EL-HARAMEIN, F. J., WILLIAMS, P. & RASHWANI, A. (1984): A simple test for the degree of damage caused in wheat by suni bug *(Eurygaster sp.)* infestation. *Rachis, 3,* 11–12.

GOTSOVA, V. & KONTEV, K. (1981): Effect of injuries caused by *Eurygaster integriceps* on the baking strength of flour of approved by zones wheat varieties. *Rastenievud. Nauki, 19,* (4), 33–43.

HIBRAOUI, M. (1930): Contribution à l'étude biologique et systématique de *Eurygaster integriceps. Revue Path. vég. Ent. agric. Fr., 17,* 97–160.

HOPF, L. (1938): Wanzenweizen. *Mühlenlaboratorium, 8,* (5), 61–67.

ISO (1981): Methods No. 520 and 712. International Organization for Standardization, Geneva, Switzerland.

KRANZ, W. (1935): Über das Auftreten der Wanzenweize bei der Ernte 1935. *Mühlenlaboratorium, 5,* (12), 177–182.

KRETOVICH, V. L. & TOKAREWA, R. R. (1943): Character and identification of injury to grain by Pentatomids. *Rev. appl. Ent., 31,* 38.

KRETOVICH, V. L. (1944): Biochemistry of the damage to grain by the wheat-bug. *Cereal Chem., 21,* 1–16.

MEREDITH, P. (1970): Bug damage in wheat. *N. Z. Wheat Rev., 11,* 49–53.

MEREDITH, P. & BEST, S. M. (1985): Studies on bug damage in New Zealand wheats. *Proceeding of 2nd. Biennial Wheat Research Institute Cereal Science Conference,* Christchurch, New Zealand, pp. 115–123.

MOHS, K. & KLEMT, G. (1936): Über die Backfähigkeit von Leimkleberweizen. *Z. ges. Getreide-, Mühlen-, Bäckereiw., 23,* (1), 1.

MITCHELL, T. A. (1971): Automatic dough mixers for high rate test baking systems. *J. Sci. Fd Agric., 22,* 626.

SNEDECOR, G. W. & COCHRAN, W. G. (1980): *Statistical methods.* 7th ed. Iowa State Univ. Press, Ames, Iowa.

Acta Alimentaria, Vol. 18 (2), pp. 211—224 (1989)

ONION IRRADIATION — A CASE STUDY

G. HÜBNER

Central Institute of Isotope and Radiation Research, 7050 Leipzig,
Permoser Strasse 15. GDR

(Received: 26 February 1988; accepted: 19 July 1988)

Onion irradiation prevents sprouting associated with long term storage. Under the climatic conditions of Central Europe, only that part of onions should be irradiated which is needed to supply the domestic market during the months of May to July. Physiological foundations of radiation-induced sprout inhibition and product-related information on cultivars, on the amounts produced, and on storage losses as well as factors influencing the efficiency of onion irradiation such as the quantity of onions irradiated during the harvest period, plant size and mechanical loading factors are presented and discussed as a result of onion irradiation during a period of seven years. National economy and business management aspects demonstrate the advantages of onion irradiation.

Two types of irradiation plants, a bulk-irradiation plant and a multipurpose large-scale irradiation plant are used for onion irradiation. Technical data, throughput, cost-related parameters of onion irradiation and benefits of the irradiation treatment are discussed. Onion irradiation for long-term storage is beneficial to the national economy as well as to the business management.

Keywords: onion irradiation, sprout inhibition, storage losses, economical aspects of irradiation

The irradiation of onions (*Allium cepa* L.) serves to prevent sprouting associated with long-term storage or transport and storage of onions in climatic conditions which stimulate sprouting. JECFI, the Joint Expert Committee for Food Irradiation of FAO/IAEA/WHO, recommended the application of an irradiation dose of up to 150 Gy for sprout inhibition with onions (FAO/WHO, 1981; 1984).

1. Physiological foundations of radiation-induced sprout inhibition

In order to determine the optimum conditions for onion irradiation, comprehensive physiological investigations were carried out (DAHLHELM et al., 1983; DAHLHELM & MATEJKO, 1984; HÜBNER, 1988). These studies showed that it is the inhibition of cell division alone which accounts for radiation-induced sprout inhibition, whereas cell elongation is not affected by ionising radiation within the range of the doses mentioned. Thus we can observe a limited growth of sprouts even after irradiation, which is caused by the elongation of meristematic cells formed by mitosis at the base of a sprout. Consequently, the most

efficient date of irradiation will depend on the stage of development and the degree of ripeness of the onions.

Our investigations have revealed that the three weeks following immediately after harvesting thoroughly ripened onions will constitute the optimum irradiation period for efficient sprout inhibition. If irradiation is carried out at a later stage, the proportion of sprouting onions will increase with long--term storage. It is not possible to extend this optimum period for irradiation by means of increased radiation doses. Having applied doses of 150 Gy, we sometimes observed an even lower sprout-inhibiting effect than within a range of up to 80 Gy (DAHLHELM & MATEJKO, 1984). Cultivar-specific differences were not observed.

Within the range of 0.05 Gy min^{-1} to 40 Gy min^{-1}, as investigated by us, the dose rate did not show any influence on the radiation-induced inhibition of mitosis. On the basis of these findings we have recommended the irradiation of onions at a dose of 30 to 70 Gy within a period of not more than three weeks after harvest.

2. Product-related information

In the GDR about 100 000 t of onions are annually produced (STATISTISCHES JAHRBUCH, 1986). The yields per hectare range between 200 and 250 decitons, and it is the special aim of this country to become self-sufficient in this field. The sowing of onions is done at the end of March, crop harvesting begins between the end of August and mid-September and has to be accomplished by October 10th at the latest. Under the climatic conditions in the GDR, onions will have reached their full and natural of ripeness by mid--September/end of September. Therefore, a so-called two stage harvesting technique is being applied (BÖTTCHER, 1970), i.e. the digging up of onions begins at a time when one-third or two-thirds of the onions leaf length are dead; and this is followed by an ultimate seven-to-ten-day windrow-ripening stage on the field. Then, these raw onions will be stored in warehouses which are equipped with outdoor-aircooling systems and owned by the cooperative farms. The weather conditions at harvest time range from sunshine to occasional showers accompanied by night dew and even fog. In September the daytime temperatures range from 15 to 22 °C, at night the temperatures are between 8 to 12 °C, the mean temperature being 14 °C, and the average rainfall 40 mm (STATISTISCHES JAHRBUCH, 1986). The main area in which onions are grown in the GDR are the countries of Magdeburg, Halle, Erfurt and Leipzig. Cooperative farms cultivating this crop reach an annual output of 2 000 t to 15 000 t of onions. In some regions a number of producers have built joint storage and processing centres with a capacity of about 16 000 t each.

Among the cultivars grown by these farms we find Früka, Zittauer Gelbe, Zerlina, Zerti, Wolska, Calbenser Gerlinde, Produskin, Makoer and Stuttgarter Riesen. The cultivar Früka ranks among the early varieties and is not suited for storage, whereas the Stuttgarter Riesen show qualities suitable to long-term storage.

During the months of May to July, about 10 000 t of onions are required to meat the demand. About 18 400 t of onions need to be produced in order to have this amount at hand. In order to prevent losses due to sprouting the onions will be prepared for wholesale in January–February, packed in bags of 20 kg, and then dispatched on pallets to cold-storage plants of wholesale organisations, where they are stored at a temperature of −2 °C before they are supplied to retail shops.

Losses at the agricultural standard stores amount to an average of 13% before the onions are moved to cold stores in February (Table 1). Before the stored products are moved from there between the end of April and the end of June, a further loss of about 17% will occur, and this will be added to losses at the retail shops, large-scale consumer centres and in households — due to an accelerated sprouting process of the onions after their removal from the cold stores. Those losses may well be in the range of 25% and above, as the increased transpiration process associated with sprouting is accompanied by higher spoilage (PETERS et al., 1984), so that the overall losses of the crop yield may amount to 45% during the whole period of long-term storage (PETERS, 1983).

Producer prices are graded according to quality and size standards (quality standards A, B, C; size standards 1 and 2; and they will rise with

Table 1

Long-term storage losses with unirradiated onions for a marketable quantity of 10 000 t

Crop volume		18 500 t
— 13 percent losses at aircooled storage by end of February	2 400 t	
Transfer to cooling sites		16 100 t
— 17 percent losses in cold stores facilities	2 700 t	
— 27 percent total losses by end of June	5 100 t	
Removed from stores by end of June		13 400 t
— 25 percent losses at trading centres and customers' places	3 350 t	
Total losses		8 450 t (45% of the crop yield)
Quantity delivered to final consumers		10 050 t

Table 2

Profits on medium-grade and medium-size onions in relation to the date of sale
(size standards 1: 70%; 2: 30%)

Month(s)	Period (calendar week)	Grade	Profit (M per t)
February/March	9th to 10th	75% A, 10% B, 15% C	1 026.50
April/May	17th to 18th	72% A, 13% B, 15% C	1 351.90
May/June	21st to 22nd	72% A, 13% B, 15% C	1 499.70
June	23rd to 24th	70% A, 10% B, 20% C	1 519.50
June/July	25th to 26th	68% A, 8% B, 24% C	1 541.40

the duration of storage. If the onions show a medium composition as regards grade and size, the proceeds will increase as shown in Table 2 from the 9th to the 26th calendar week (LUTHER, 1986).

3. The irradiation of onions

3.1. National economy aspects

Since 1981 more than 2800 t of onions have been irradiated at a pilot plant in the GDR. A newly built industrial plant irradiated 4675 t in 1986, which means that our reports are based on experience gained with about 7500 t of irradiated onions at two plants of different types. Only that proportion of onions is envisaged for irradiation, which is required for the market during the months of May to July, because the prevailing climatic and storage conditions will not bring about any differences in the sprouting behaviour of irradiated and unirradiated onions before April. Due to prolonged storage, the onions envisaged for irradiation must meet higher quality standards in order that additional losses are avoided which might be caused by spoilage resulting from larger amounts of damaged onions. This also implies that there should not be any further damage induced by transporting the onions to the irradiation plant, within the same and from there to the storage sites.

Bearing in mind that irradiation must be performed not later than three weeks after harvesting, it should normally be an integral part of the harvesting and storage processes to guarantee optimum sprouting inhibition. Due to the inhibition of sprouting the irradiated onions need not be moved to cold-storage plants, so that they can be stored as raw produce at their farm stores till treatment for sale. This means shifting the activities necessary for preparing the onions for the market from February/April to March/June. The period from April to June, however, is characterized in agriculture by a high

labour demand for numerous other jobs to be carried out on the fields. This means that the procedure of irradiation has to be adapted to farm-management possibilities, in order that the irradiated onions can be sold at a time which is most suitable and promises the best possible reward in terms of national economy.

Storage and marketing experiments with irradiated onions show that the losses at outdoor-air-cooled stores of the farms are clearly below 20%, which compared favourably to 27% occurring with cold storage of high-grade onions. When damaged, wet or not thoroughly ripened onions are irradiated, the losses during storage can be in excess of 30% (LUTHER, 1986). Sprout inhibition will result in a significant reduction of losses in wholesale and

Table 3

Losses and energy requirements with long-term storage of unirradiated and irradiated onions

	Cold stored	Irradiated
Crop volume	18 500 t	13 000—15 000 t
— Losses due to storage by end of June	5 100 t	2 500— 4 500 t
— Losses at the trading centres and on the customers side	3 500 t	500— 500 t
— Total losses	8 500 t (46%)	3 000— 5 000 t (23—33%)
Marketable quantity	10 000 t	10 000 t
Energy required for cold storage[a]	332 kWh t^{-1}	
cold storage of 16 100 t	5 345 MWh	
Energy required for irradiation and storage with ventilation system[a]		53 kWh t^{-1}
Irradiation of 15 000 t		795 MWh

[a] according to PETERS (1983)

Table 4

Material and services savings with the supply of 10 000 t of irradiated onions

Reduced volume of production	3 000—5 000 t of onions
Cultivated area (x = 220 dt/ha)	130—225 ha (4% of the area cultivated with onions)
Labour hours (production on the fields, processing)	36 000—60 000 manpower hours
Seeds	1.5—2.5 t
Plant protection agents	5—9 t
Fertilizers	40—70 t
Diesel fuel (work on the fields)	28.5—50 t
Transport requirements	350 000—600 000 tkm
Energy requirements	4 600 MWh (1.1 Mio Mark)

retail trade as well as with the consumers so that the amount of onions supplied to customers will be increased by irradiation, whereas the total losses will be reduced. Based on a quantity of 10 000 t of onions supplied to the customers, the values shown in Table 3 were obtained. If 10 000 t of irradiated onions are made available, the lower losses will result in significant savings in terms of national economy (Table 4). Savings of 4600 MWh of electric power alone are equivalent to M 1.1 million, which can be saved by eliminating cold storage, which means that a price of 75–85 M/t for irradiation will make this technique profitable by energy savings alone.

3.2. Business management aspects

Seen from the business management's point of view, the higher cost of irradiation as well as the longer duration of storage and the shifting of labour peaks for marketing to the months of April and May have to be taken into account. The prolonged duration of storage means higher risks, because the rates of losses will increase with outdoor-air-cooled storing. The capacities available for processing and marketing the onions at the enterprises are matched to the volume of production and aim at a stable marketing period from mid-September to May. Thus, only part of the total onion crop can be made available by the enterprises in the period of May to July. This proportion will be determined by the shelf life of the irradiated onions prepared for the market and by the availability of labour at the time of onion marketing in May.

PETERS (1983) reports that irradiated onions can be stored an additional four to six weeks at the production enterprise after cleaning, size classification and packing into bags of 20 kg, provided that forced-air ventilation systems are employed. In terms of quality there will only be insignificant losses with well-graded onions. This makes possible the sale of the products to trading organisations without any additional sorting stages. LUTHER (1986) was able to prove that well-treated onions, stored in bulk intermediately, did not show any losses of mass in excess of 4.5% even after 8 weeks, and that the commodity could be marketed without any significant expenditure on re-separation. By mid-March or (according to LUTHER, 1986) at the beginning of March the processing of irradiated onions could be started and thus 25 to 35% of the total working hours for the processing activities could be made available for marketing the irradiated onions. In other words, a production volume of 2000 t would mean marketing activities for irradiated onions in the range of 500 to 700 t, and with a production capacity of 15 000 t, 3500 to 5000 t of irradiated onions could be marketed.

The principles of crop rotation allow the growth of onions on the same patch of land only every seven years, i.e. medium-range yields of 220 dt ha^{-1}

require farm size of 750 to 1500 ha for the production of 2000 to 4000 t of onions. With crop yields of 15 000 t per annum, the minimum farming area would have to be 5000 hectare.

Mainly due to the weather conditions, there are 20 working days available to collect the crops. For the driest possible storage of the onions they should not be collected before the dew has disappeared, i.e. there will be a potential working time of about 10 h per day. With crop yields of 15 000 t this means a storage capacity of 75 t per hour. Extension of the daily warehousing times could result in higher expenses for drying the onions or minor onion qualities, which would mean increased losses during the storage period by spoilage. Longer additional transport and intermediate storage periods of the onions to be irradiated have to be avoided in order to prevent possible damage and subsequent higher losses at the warehouses. Hence, the period of irradiating the onions is nearly identical with that of harvesting.

The daily time of irradiation can be extended to 15 h if the onions collected from the field in the evening remain on their respective transport vehicles until they are irradiated. This would result in a harvesting season of 200 to 300 h, which, in turn, would require a plant capacity of 3.5 t h^{-1} to irradiate 700 t of onions. A throughout of at least 14 t h^{-1} is required to irradiate the total amount of 4000 t.

4. Irradiation facilities for onions

In 1981, a pilot plant for the irradiation of onions was put into operation at the Weideroda onion store of the farming cooperative Wiederau-Zwenkau (county of Leipzig) (DÖLLSTÄDT et al., 1983; HÜBNER et al., 1984). The plant

Table 5

Specifications of the GBZ 81 bulk-type irradiation plant

	Pilot plant	Commercial plant
Cobalt-60	1.5×10^{15} Bq (40 kCi)	1.85×10^{15} Bq (50 kCi)
Throughput	4–5 t h^{-1}	7–8 t h^{-1}
Transport system	turn table	turn table
Layer of onions	60×20 cm	80×40 cm
Overdose ratio	2.3	2.3
Dose	30–70 Gy D = 45 Gy	30–70 Gy D = 45 Gy
Mean dose rate	70 Gy min^{-1}	55 Gy min^{-1}
Throughput per harvesting season	800–1 000 t	1 500–2 500 t

consists of an underground bunker with a turn table of 4 m in diameter. A layer of onions, 60 cm wide and 20 cm high, passes the cobalt-60 radiation sources located in two tubes each below and above the layer of onions. Conveyor belts transport the onions to and from the plant. Plant specifications are given in Table 5. This bulk-irradiation plant handled 195 t in 1981 and since 1982 the annual throughout has risen to 500–600 t of onions. Due to the positive results achieved the plant is planned to be redesigned into a commercial scale one with an increased throughput (Table 5). The capacity would then be in the range of 1800–2400 t of irradiated onions per harvesting season. In terms of business economy such a plant would be profitable with crop yields of about 6000 t of onions.

The bulk-irradiation plant is an integral part of the onion storage line linking the field site with the outdoor-air-cooled storage facility of the agricultural enterprise. According to the findings of a long-term analysis of the Weideroda pilot plant, the additional dropping and transport stages will lead to an increased proportion of damaged onions. The increase amounts to 3–5%. Experience will be taken into account when this plant is redesigned in order to minimize the mechanical loading factors of the crop by employing slow-running conveyor belts, by reducing the dropping heights at transfer points and by a continuous flux of products through the plant.

At the Spickendorf onion-processing branch of the farming cooperative Queis (county of Halle) a multi-purpose large-scale irradiation plant was commissioned in 1986. Its irradiation capacity is approximately 4000 t of onions

Table 6

Specifications of the GBS 84 multi-purpose large-scale irradiation plant

Cobalt-60	1.11×10^{16} Bq (300 kCi) 1.85×10^{15} Bq (50 kCi) for onion irradiation
Throughput	512 kg×10 kGy h^{-1} maximum of 18 t of onions per h
Transport system	roller conveyor (computer-controlled)
Container sizes	$1.00 \times 1.20 \times 2.70$ m^3 $0.95 \times 1.15 \times 1.95$ m^3 (for onions
Maximum weight	1 t per unit
Overdose ratio	1.5 (at $f = 0.3$ g cm^{-3}) (2.3 for onions)
Availability	7 000 h/a
Use for onion irradiation	700 h/a
Capacity per year	3 250 t×10 kGy 4 000 t of onions

per harvesting season. Off-season operation at the computer-controlled plant comprises the treatment of a wide range of products with differing doses of radiation.

The products for irradiation pass this plant in containers, boxes or on pallets with a maximum weight of 1 t. The product-handling units pass the plant on roller conveyors and are exposed to four-side irradiation from a cylindrical radiation source. The plant specifications are given in Table 6 (HÜBNER, 1984).

For irradiation the onions are filled into the containers by an automated loading mechanism, which is an integral part of the storage line. After passing the plant they are discharged. Container transport is performed at a computer-controlled cycle time of 2 min 50 sec and this permits a maximum throughput of 18 t h^{-1}. Taking into account potential disturbances occurring at the feeding and transport systems, an average throughput of 15 t h^{-1} can be processed. Thus 3500 to 4500 t of onions can be irradiated per harvesting season. During the trial run of the plant in the 1986 harvesting season a total of 4675 t of onions were treated during a radiation time of 378 h, and this is equal to an average throughput of 12.4 t h^{-1}.

5. Cost-related parameters of onion irradiation

For the erection and equipment of a GBZ-type bulk-irradiation plant (without cobalt-60) expenses have to be planned at about M 1 million (HÜBNER, 1988). Depreciation, interest, rates spendings on labour and material as well as other services require an annual expenditure of M 131 300 on the operation of this plant, if it is only used during the time of harvesting the onions. To this the costs for the radiation sources have to be added, which depend on the price of cobalt-60 and the annual operating time of the sources (Table 7). The values

Table 7

Expenditure on 1.85 × 10^{15} Bq (50 kCi) of cobalt-60 in relation to the price of cobalt-60

	5 M per Ci	10 M per Ci	25 M per Ci	46 M per Ci
50 kCi	250 000	500 000	1 250 000	2 300 000
12.6% for recharging per year	31 500	63 000	157 500	289 800
1.6% depreciation per a	4 000	8 000	20 000	36 800
0.18% insurance	450	900	2 250	4 140
5% interest	12 500	25 000	62 500	115 000
19.38% overall expenditure per a	48 450	96 900	242 900	445 740
Proportional costs for 6 weeks	5 570	11 150	27 850	51 260

Table 8

Irradiation costs per ton of onions at the GBZ 81 bulk-type irradiation plant in relation to the price of cobalt-60 and to the quantity of irradiated onions

Price of Co-60 (M per Ci)	Overall costs per year	1 000 t (M per t)	1 800 t (M per t)	2 400 t (M per t)	3 000 t (M per t)
5	136 870	137	76	57	46
10	142 450	143	79	60	47.50
25	159 150	160	88	66	53
46	182 560	183	101.50	76	61

Table 9

Costs per operating hour at the GBS 84 large-scale irradiation plant loaded with 7.4 PBq (200 kCi) of cobalt-60

Co-60 price (M per Ci)	Co-60 costs per year	Annual overall costs (in 1 000 M)	Costs per operating hour (7 000 h per a)	Costs for onion irradiation (700 h)
5	193 800	1 218.8	174	121 900
10	387 600	1 412.6	202	141 300
25	1 782 960	2 808.0	401	280 800
46	2 325 600	3 350.6	479	335 100

clearly indicate that it is desirable to employ the radiation sources not only during the time of the onion harvest in order to keep the costs induced by the price of cobalt-60 as low as possible.

In dependence on the price of cobalt-60 and on the quantity of onions irradiated during one harvesting period, the irradiation prices of the bulk--irradiation plant will vary from M 50 to 100, if at least 1800 t of onions will be irradiated per harvesting season (Table 8).

Erection and equipment of the GBS-type large-scale irradiation plant (without cobalt-60) require an expenditure of about M 6 million (ANON., 1988). The annual operating costs (without cobalt-60) amount to M 1 025 000 with a potential load of 7.4×10^{15} Bq (200 kCi) of cobalt-60. Dependent on the price of cobalt-60, we have to add annual expenses for the irradiation sources, so that costs per operating hour will arise which are based on 7000 operating h per year (Table 9).

Due to the duration of the harvesting season a period of 4 weeks has to be envisaged for the irradiation of onions, which result in planning 700 h for the irradiation of onions. Dependent on the total amount of irradiated onions, the irradiation costs per ton vary from M 30 to 90 (Table 10).

Expenditure and prices for the irradiation of other products at the GBS 84 large-scale irradiation plant are given in another paper (HÜBNER, 1988).

Table 10

Costs for onion irradiation at the GBS 84 large-scale irradiation plant in relation to the price of cobalt-60 and to the quantity irradiated during one harvesting season

(loading with multi-purpose application: 7.4 PBq Co-60)

Price of Co-60 (M per Ci)	Irradiation costs per ton (M)			
	3 000 t	3 500 t	4 000 t	4 500 t[a]
5	40.60	34.80	30.50	27
10	47.10	40.40	35.30	31.40
25	93.60	80.20	70.20	62.40
46	111.70	95.70	83.80	74.50

[a] quantity irradiated during one harvesting season

The data presented make it possible to derive two examples of the cost/benefit ratio of the irradiation of onions for the farms:

— a farm with a crop volume of 6000 t of onions irradiates 18 000 t at a bulk-irradiation plant for marketing during the period between May to July;

— a farm with a crop volume of 15 000 of onions irradiates 3500 t at a large-scale irradiation plant.

The calculations are based on the following assumptions:

— onion processing is performed during the period from mid-September to May;

— irradiated onions are processed in the period from March to May, but they will be dispatched to an intermediate store for 8 weeks before being marketed in the period from May to July;

— unirradiated onions have to be marketed immediately after processing;

— storage till May results in losses of 20%, and storage till July in 25%;

— from May onwards, an additional price of M 90 per ton of irradiated onions will be paid;

— irradiated onions cause additional storage and processing costs of 10 M per ton;

— the price of cobalt-60 is 5 or 25 M per Ci;

— the large-scale irradiation plant is loaded with 7.4 PBq (200 kCi) of cobalt-60.

Taking these assumptions into account, the additional profits mentioned in Table 11 will be gained for the irradiated onions. From these calculations it results that an additional profit of M 140 per ton of irradiated onions will be made at both types of plants with the quantities assumed for the irradiation of onions and with a cobalt-60 price of 25 M per Ci.

Table 11

Additional proceeds for irradiated onions with a quantity of 1800 t (at the GBZ 81 plant) *or respectively, 3500 t* (at the GBS 84 plant)

	Bulk-type irradiation plant		Large-scale irradiation plant	
Quantity harvested	6 000 t	6 000 t	15 000 t	15 000 t
irradiated	1 800 t		3 500 t	
processed by end of February	4 000 t	4 000 t	10 000 t	10 000 t
processed from March to May (20% losses)	200 t[a]	2 000 t	1 500 t[a]	5 000 t
	1 800 t[b]		3 500 t[b]	
Proceeds from March to May (1 200 M/t) (in 1 000 M)	192	1 920	1 440	4 800
Sales from March to May (20% losses)	160 t	1 600 t	1 200 t	4 000 t
Sales from May to July (25% losses)	1 350 t		2 625 t	
Proceeds by July (1 500 M/t + 90 M/t)	2 146 500 M		4 173 750 M	
Additional costs for storage and processing	13 500 M		26 250 M	
Overall proceeds from March to July	2 325 000 M		5 587 500 M	
Additional proceeds with irradiated onions		405 000 M		787 500 M
Radiations costs	5 M/Ci	137 000 M		122 000 M
	25 M/Ci	160 000 M		281 000 M
Profit	5 M/Ci	268 000 M (150 M/t)		665 500 M (190 M/t)
	25 M/Ci	245 000 M (136 M/t)		506 500 M (144 M/t)

[a] unirradiated
[b] irradiated

Any increase of the amount of irradiated onions will improve this result, provided that the quality of the stored onions can be maintained.

The data of Tables 8 and 10 indicate that in the case of the bulk-irradiation plant the irradiation price is mainly determined by the installation costs of the plant, whereas at the large-scale irradiation plant, which is in operation all the year round, the expenditure on cobalt-60 will mainly influence the price of irradiation. Since only part of the existing cobalt-60 at the large-scale irradiation plant will be used for irradiating the onions, the remaining portion could be leased to three bulk-irradiation plants to be used there during the harvesting season. Charging a price of M 20 000 per 1.85 PBq and

a leasing period of four weeks would reduce the costs spent on onion irradiation at the large-scale irradiation plant by M 60 000.

The philosophy of meeting the demand for onions in the months of May to July and of a simultaneous substitution of the cold storage calls for a combination of one large-scale irradiation plant with three or four bulk--irradiation plants. Using such a plant combination ensures the irradiating and marketing capacities necessary for 10 000–12 000 t of onions at decentralized locations.

Outside the harvesting season, the total amount of cobalt-60 at all these plants can be used at the multi-purpose large-scale plant to irradiate a wide range of other products. Consequently, all the plants have to pay only proportionate shares for using cobalt-60 and, in turn, this will keep the irradiation expenditure on onions below 75 M per t in spite of the high prices for cobalt-60. Hence, the irradiation expenses for 13 000 t of onions will amount to a total of M 975 000, which is less than any expenditure on power that would have to be spent on storing the same amount of marketable unirradiated onions in cooling facilities.

As the benefits of an irradiation treatment and a subsequent reduction of losses occurring during the storage period will be mainly on the trading organisations — and the customers — sides, the charging of an additional 90 M per t of irradiated onions by the producing farms is justified. In conclusion it can be stated that the procedure of irradiating onions for long-term storage is beneficial to the national economy as well as to the business management.

Literature

BÖTTCHER, H. (1970): Untersuchungen zur Gestaltung des komplexen Systems der Ernte, Bergung und Nacherntebehandlung von Speisezwiebeln. *Dt. Gartenb., 17*, 214–218.

DAHLHELM, H., MATEJKO, C. & HÜBNER, G. (1983): Experiments on sprouting inhibition in onions. *ZfI-Mitteilungen, 69*, 395–407.

DAHLHELM, H., & MATEJKO, C. (1984): The mitotic activity of onions during the storage period in relation to their radiosensitivity. *ZfI-Mitteilungen, 98*, 655–666.

DÖLLSTÄDT, R., GRAHN, CH., HÜBNER, G. REINHARDT, J., SCHULZE, W. H. SPRINZ, H. & WINKLER, E. (1983): Irradiation Facility GBZ 81. *ZfI-Mitteilungen 70*, 178–186.

FAO/WHO (1981): Wholesomeness of irradiated food. Report of a Joint FAO/WHO Expert Committee. World Health Organisation Technical Report Series, Geneva

FAO/WHO (1984): Codex general standard for irradiated foods. Vol. XV. ed. 1. Codex Alimentarius Commission, FAO/WHO, Rome.

HÜBNER, G. (1984): A new multipurpose gamma irradiation facility. *ZfI-Mitteilungen 98*, 578–589.

HÜBNER, G. (1988): Strahlenbehandlung von Speisezwiebeln. *ZfI-Mitteilungen, 138*, 5–215.

HÜBNER, G., WINKLER, E., SCHÜMICHEN, P. & LUTHER, TH. (1984): Process engineering aspects and the further development of the onion irradiatior GBZ 81. *Zfl-Mitteilungen, 98*, 606–617.

LUTHER, TH. (1986): Nuklidbestrahlungsanlage für Dauerzwiebeln *(Allium cepa* L.) unter besonderer Berücksichtigung der Nebennutzung. Diplomarbeit. Martin-Luther-Universität, Halle–Wittenberg.

PETERS, P. (1983): Entwicklung eines Verfahrens der Keimhemmung und Haltbarkeitsverlängerung von Speisezwiebeln in der LPG Wiederau/Zwenkau auf der Grundlage einer Bestrahlung mit Cobalt-60. Forschungsbericht des Instituts für Gemüseproduktion.

PETERS, P., HÜBNER, G., NEBE, D. & ZEUTSCHEL, K. H. (1984): Bestrahlung von Speisezwiebeln mit Co-60 zur Verlängerung der Lagerdauer im Normallager und Qualitätssicherung bis zum Endverbraucher. *Gartenb.*, *31*, 259–263.

STATISTISCHES JAHRBUCH (1986): Statistisches Jahrbuch der Deutschen Demokratischen Republik. Staatsverlag der Deutschen Demokratischen Republik, Berlin, p. 14 9

Acta Alimentaria, Vol. 18 (2), pp. 225—243 (1989)

RECENT ADVANCES IN FLAVOUR RESEARCH USING CHEMOMETRICS

T. AISHIMA

Noda Institute for Scientific Research, 399-Noda, Noda-shi,
Chiba-ken 278. Japan

(Received: 30 March 1988; accepted: 27 August 1988)

Chemometric methodologies, especially multivariate analysis, are playing an ever increasing role in correlating sensory evaluation to instrumental data. Sensory evaluation and instrumental analysis using GC, HPLC, GC-MS and GC-FTIR clearly demonstrated the intrinsic multidimensionality of flavour. The application of multivariate analysis to analyzing flavour data is categorized: correlating instrumental measurements to sensory evaluation, sample discrimination, extraction of information from data matrix and sample clustering. Successful results obtained from multivariate analysis have been contributing to establish objective evaluation of flavour quality. Simplex method is the most widely used optimization algorithm in chemometrics. The conditions to produce the most desirable strawberry juice flavour was easily be found by simplex optimization in a multivariate parameter system. Recent trends in flavour research are reviewed by especially focusing on the utilization of chemometrics.

Keywords: chemometrics, flavour, multivariate analysis, simplex optimization

Flavour analysts have been focusing their efforts on finding the causal relationship between instrumental analysis and sensory evaluation in order to establish the objective assessment system of flavour (MARTENS et al., 1987). Once we succeeded in establishing a system describing flavour difference or preference based on chemical components, quality control, production control and flavour design for new products which meet customers tastes can efficiently be performed. Results from multivariate statistical analysis of texture profiles obtained both from texturometer analysis and from sensory evaluation using the "texture profile" method were promising (STANLEY et al., 1988; SZCZENIAK, 1987). However, most of the prominent advances have been shown in the analysis of volatile flavour (MARTENS & HARRIES, 1983).

From the view point of volatile flavour, instrumental analysts have mainly focused their efforts on finding a causal relationship between single or a group of a few compounds and a specific food property (BUTTERY, 1981). However, naturally, most of such attempts failed due to the intrinsic complexity or multidimensionality of flavour. Since the late 60's, propagation of computer and chromatographic analysis opened new era for flavour analysis (POWER & KEITH, 1968). In the mid 70's "Chemometrics", a new interdisciplinary area, was originated in analytical chemistry by merging computer science and instru-

mental analysis (KOWALSKI, 1977). Pattern recognition, a major element of chemometrics, is now showing the great capability for handling multivariate data sets derived from both sensory evaluation and instrumental analysis of flavour components (DRAVNIEKS, 1974; POWERS, 1982; AISHIMA, 1983). Simplex method is the most widely used optimization algorithm among analytical chemists (DEMING & MORGAN, 1983). However, the simplex optimization is still far from popularity among neither food scientists nor food technologists in spite of its easy theory and wide versatility (SAGUY et al., 1984). In this article applications of chemometric methodologies to flavour evaluation and production optimization are reviewed.

1. Flavour

Flavour is perceived through our five senses such as sight, hearing, touch, smell and taste (HALL, 1968). Detections of taste and smell are performed through the perception for low molecular chemical compounds, therefore called chemical senses. On the other hand, sight, touch and sound are all physical senses because these three senses detect physical properties of foods.

Thus flavour may be explained as a linear combination of weighting factors and sensory observations. Actually the relationships among properties are not so simple due to the mutual interaction existing among them: e.g., temperature and texture are mutually correlating and affecting the observation of other sensory properties. Naturally, the weighting factors for each property are different from food to food. Aroma is one contribution to flavour. It may also be explained as a linear combination of weighting factors and several aroma compounds. Of course mutual interaction known as synergy or compensation takes place among components but the accurate calculation by taking account such interaction is still far beyond the capability of today's most advanced computers due to the astronomical number of possible combinations of flavour components in foods. Furthermore, nature and kind of such interactions in most cases is virtually unknown. Resulting data from sensory evaluation and chemical analysis may be combined in matrices of high dimensionality. Assuming a causal relationship, variables can be extracted from such matrices, called predictors and criterion variables. Values are attributed to these variables by regression analysis on sets of experimental observations.

2. Sensory evaluation

Sensory evaluation is classified based on their methodologies and objectives (Fig. 1) (PANGBORN, 1984). Sensory evaluation utilizes human senses as the transducers to quantify stimuli (AMERINE et al., 1965). Selection and training of panel members for sensory tests should be standardized to obtain reliable results from them (SENSORY EVALUATION DIVISION of IFT, 1981; ASTM COMMITTEE E-18, 1981). Nominal, ordinal, interval and ratio scales are used to express stimuli (STEVENS, 1946). Interval and ratio scales are metric and therefore normal arithmetic calculation such as analysis of variance or regression analysis is directly applicable. On the other hand nominal and ordinal scales are nonmetric and therefore ordinary arithmetic handling should not be applied. Comparison between interval and ratio scales had been the point of an argument among sensory analysts regarding accuracy in the quantification of flavour difference. However, no significant difference was found between the results obtained by using two scales when well trained panel was applied to the test (MOSKOWITZ & CHANDLER, 1977). Number of categories had also been the second point of an argument. Better linearity was obtained using smaller category number while better discriminative efficiency was shown by larger category numbers (JONES et al., 1955).

Quantitative descriptive analysis (QDA) is the most informative sensory evaluation so far (STONE et al., 1974; MECREDY et al., 1974) although this method requires well trained and experienced sensory test panels. QDA provides key factors deciding the flavour quality and quantitative difference

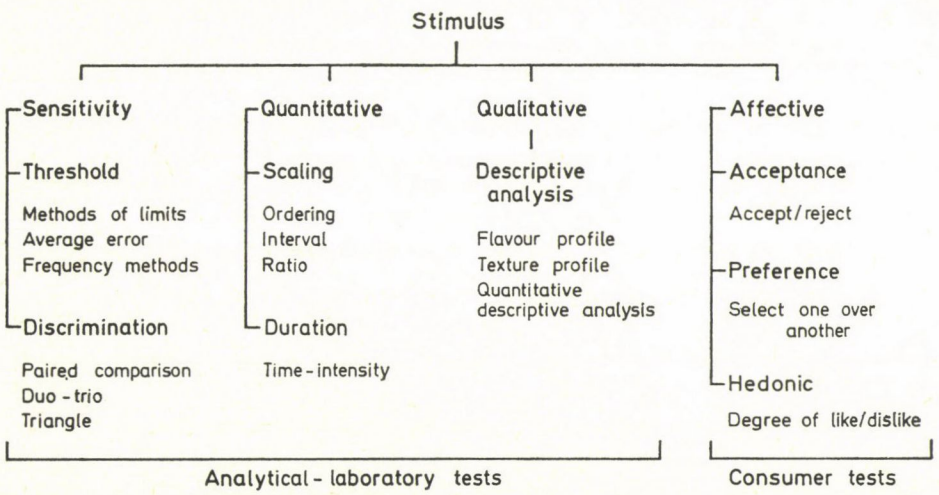


Fig. 1. Classification of sensory evaluation

of these properties among samples. If we have these sensory data for the samples in the problem, these data may easily be correlated to the instrumental data.

3. Instrumental analysis

3.1. Gas chromatography (GC)

GC and GC-MS (mass spectrometry) analyses have commonly been applied for volatile flavour analysis. Aroma analysis is composed of three different steps (TERANISHI et al., 1981), i.e., extraction and concentration of volatile components from food matrix; quantitative analysis using GC and identification or structure elucidation of components by hyphenated instruments such as GC-MS, LC(liquid chromatography)-MS and GC-FTIR (Fourier transform infrared spectrometry). Dynamic headspace analysis or purge and trap method using porous polymer such as Tenax and Porapak (NUMEZ & GONZALEZ, 1984), simultaneous distillation and extraction (SDE) method using a modified Likens-Nickerson's apparatus (FLATH & FORREY, 1977) and supercritical fluid extraction mainly using carbon dioxide (KRUKONIS, 1985) are the most popular three methods among flavour analysts.

Today many different GC detectors are available. Structurally differential GC analysis is possible only by using GC because each detector responds to specific atoms (DRUSHEL, 1983). However, the most conspicuous progress was given in GC column technology. The fused silica capillary column on which liquid phase is coated with covalent bond may almost be the ideal column expected so far.

3.2. Hyphenated instruments

Recently, identification of flavour components in foods became an easier task due to the development of hyphenated analytical instruments. Until recently only GC-MS was the hyphenated instrument used widely in flavour field. However, now, FTIR coupled with GC is showing advantage over GC-MS in identifying positional isomers which can not be discriminated due to their similarity of MS spectra (HERRES, 1984). Every hyphenated instruments are supported by a computer system which comprises a large spectrum data base and library search algorithm for easy interpretation of data. Artificial intelligence started showing great capability for the data interpretation both in MS and IR spectra (WOODRUFF, 1984).

4. Chemometrics

4.1. Chemometrics

Chemometrics was defined as "the application of mathematical and statistical methods to design or select optimum procedures and experiments and to provide maximum chemical information by applying chemical data" (KOWALSKI, 1981) and has been very rapidly developing in analytical chemistry since the mid 70's. The progress of chemometrics has been accelerated by the advances both in instrumental analysis and in computer technology. According to Kowalski's group, chemometrics compose of eleven different elements such as statistics, optimization, modeling and parameter estimation, calibration, factor analysis, resolution, signal processing, pattern recognition, library searching, structure–property relationships and artificial intelligence (RAMOS et al., 1986). Most of the mathematical and statistical methods in chemometrics are applied to multivariate data sets. Multivariate analyses are therefore very popular in chemometrics. The reason why analytical chemists or environmental analysts introduced chemometrics into their area might be due to the complexity and diversity of their objects (BREEN & ROBINSON, 1985). The reason of necessity for applying chemometric methodologies to flavour analysis is exactly the same for flavour analysts: that is to say, the complexity and diversity of flavour components.

4.2. Advantage of higher dimensionality

In spite of the intrinsic multidimensionality of flavour, most of statistical analyses have been carried out by utilizing univariate or bivariate methods such as analysis of variance and regression analysis. Advantage of higher dimensionality lies in the simultaneous judgement of a higher number of complex factors. It is payed for by a loss of human imagination, which is restricted to a low number of dimensions. Through the development of adequate computer technologies, computational techniques became easier and available anywhere. Furthermore, it can serve to extract factors and influences and to reduce it to a picture of lower dimensionality. The contents of which can be perceived by human imagination again.

4.3. Books and softwares

Several books including standardized textbooks (MASSART et al., 1987; VARMUZA, 1980; DEMING & MORGAN, 1987) and reviews (DEMING, 1986; FISHER & JONES, 1987; FORINA et al., 1987) on chemometrics in different fields have been published. Two journals, Elsevier's "Chemometrics and Intelligent Laboratory Systems" and Wiley's "The Journal of Chemometrics", have been published since 1986 and 1987, respectively. Many different soft-

wares are available for main frames, mini computers and personal computers. General software sets which are composed of many multivariate analyses such as BMDP (biomedical computer programs) (DIXON, 1985), SAS (statistical analysis system) (RAY, 1985) and SPSS (statistical package for social sciences) (NORUSIS, 1985), are also widely used by flavour analysts and elsewhere in analytical and social sciences. Arthur, Simca and PLS (names of program packages discussed later) include various pattern recognition programs and are mainly distributed among chemometricians. Publishing companies are releasing chemometric softwares including educational programs for laymen such as Cleopatra of Elsevier Scientific Software.

5. Pattern recognition

The relationship between the sensory evaluation and instrumental analysis of flavour is shown in Fig. 2 (AISHIMA & NOBUHARA, 1977). If these two matrices are correlated to each other by using chemometrics, then it means that we can integrate both data sets and eliminate duplicate, since correlated, variables (dimensions). Hence, the flavour quality can be assessed independently from the sensory test panel thereafter. The data matrix A is obtained from sensory evaluation while the data matrix B is brought from instrumental analysis, sometimes data transformations being applied to linearize or standardize distributions of variables. Both matrices are connected by using multivariate analysis.

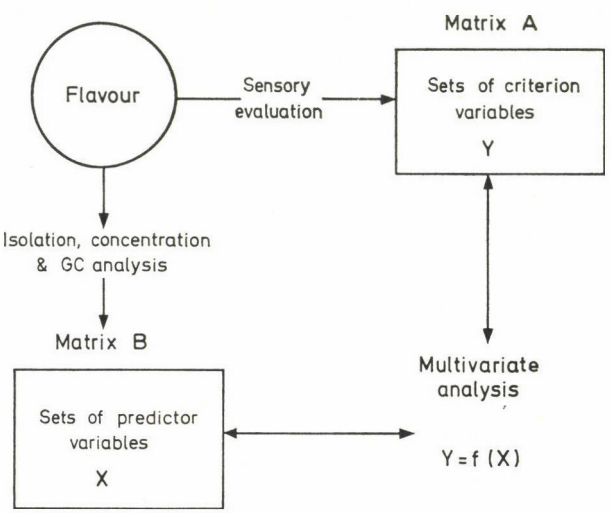


Fig. 2. Correlating sensory evaluation to chromatographic analysis of flavor components using multivariate analysis

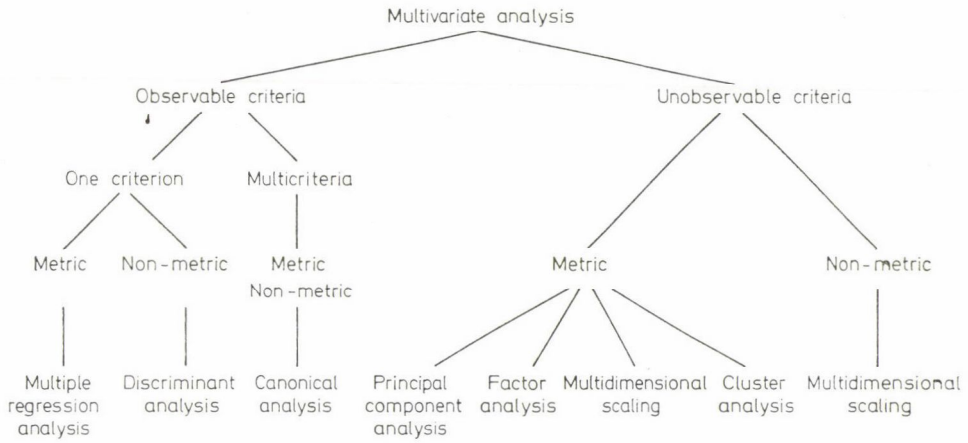


Fig. 3. Taxonomy of multivariate analysis

As easily understandable, multivariate analysis has great advantage over univariate analysis. So far many different multivariate analyses and their programs have been applied to flavour analysis. Those methods are classified based on whether variables are metric or nonmetric, single criterion or multicriteria and observable or unobservable criteria (Fig. 3). Seven methods shown in Fig. 3 have frequently been applied to flavour analysis. Recently other multivariate statistical methodologies such as partial least squares (PLS) analysis (MARTENS & MARTENS, 1986) soft independent model class analogy (SIMCA) (DERDE et al., 1984), multidimensional scalling (MDS) (SCHIFFMAN et al., 1981) and procrustes analysis (ARNOLD & WILLIAMS, 1986) were getting popularity among flavour analysts. However, MDS and procrustes analysis have only been used for sensory data. Number of application of PLS for multivariate calibration is particularly increasing due to its capability (MARTENS, 1985). Comprehensible books concerning multivariate statistical analysis in food research were published (MARTENS & RUSSWURM Jr., 1983; PIGGOTT, 1986).

5.1. Correlating instrumental measurements to sensory evaluation

Multiple regression analysis (MRA) has widely been applied to correlate single metric criterion to multimetric predictors in order to calculate multiple regression models to predict flavour quality (SIMON et al., 1980; AISHIMA, 1981; LELAND et al., 1987). MRA is based on the ordinary least squares method, difference between them being only multipredictors in MRA. Two necessary conditions must be kept to obtain reliable results from MRA. Firstly, the number of samples should be larger than that of predictor variables. Secondly,

highly correlating variables should not be used as the predictors in order to avoid the problems occurring from multicollinearity. Ridge regression analysis was available in order to avoid this problem (HAITOVSKY, 1987), however, no applications to flavour analysis have been reported so far. If criterion variables are more than two, then canonical analysis can be applied to such data sets.

Multiple regression models were calculated from sensory data and GC data obtained using a glass capillary column (AISHIMA, 1981). At two different stages of stepwise regression procedure, coefficients of determination of 0.857 and 0.903, respectively, were obtained. These figures suggest that more than 90% of information in the sensory data was explained by the selected ten peaks. Analysis of variance clearly suggests the existence of statistically significant correlation between GC and sensory data sets. Thus we obtained good predictive equations. However, we should be careful to interprete the meaning implied in the regression models because there are two possibilities of such significance, i.e. correlation and a causal relationship.

5.2. Sample discrimination

Supervised discrimination methods such as linear discriminant analysis (LDA), SIMCA and K-nearest neighbourhood (KNN) (VARMUZA, 1980) have been used for sample classification based on chromatographic data. Among above three methods, KNN performs nonparametric discrimination. LDA and canonical analyses become the same algorithm when criterion is expressed as a diagonal matrix of dummy (1.0) variables in LDA, in which a row corresponds to a group. Accuracy of discrimination among LDA, SIMCA and KNN were compared by different groups but significant difference was not found

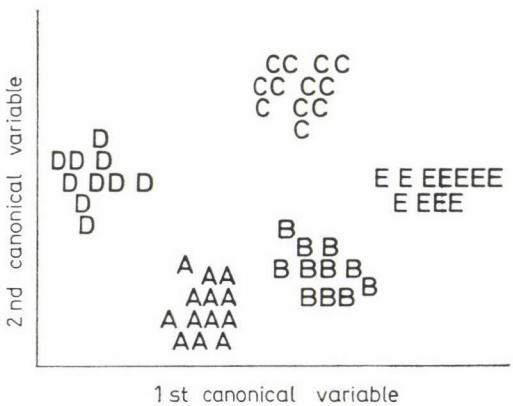


Fig. 4. Canonical plot of five brands (A-E) of Worcestershire sauce samples

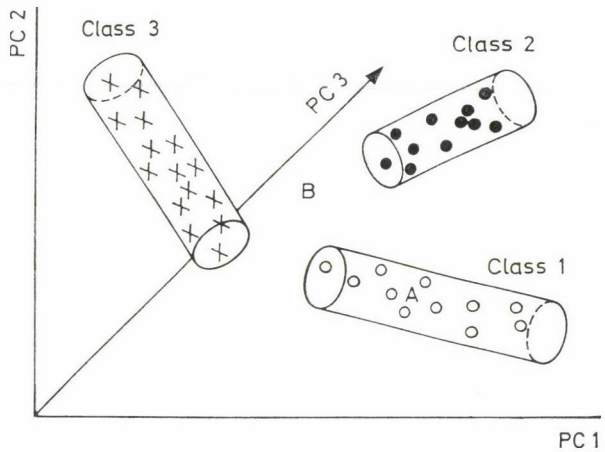


Fig. 5. Hyperboxes in SIMCA, PCl, PC2 and PC3 stand for principal components. Tolerance regions have been constructed around each class (cylinders) on the basis of the scatter of the training set points around the models. Sample A is assigned to class 1; sample B is an outlier

among them (FORINA et al., 1986; LARRECHI et al., 1987). Application of LDA to GC or HPLC data sets of many different foods has been reported.

The discrimination of Worcestershire sauce using BMDP7M program based on their GC data is shown in Fig. 4 (AISHIMA, 1986). Worcestershire sauce is one of the most popular seasonings in Japan. Every brand seems to have its own unique formulation of spices in order to differentiate flavour of their products from competitors. However, GC profiles of five brands were too complicated to differentiate them by comparing their patterns. Exclusive sample clusters derived from five brands were observed on the first and second canonical variables. Canonical analysis is used in analyzing one set of predictor variables and the other set of criterion variables simultaneously. When only one criterion is available, canonical analysis reduces to multiple regression analysis. Samples belonging to the five brands could almost perfectly be assigned using the combination of several peaks. If a number of i peaks were needed to discriminate samples, then it means that samples were discriminated in an i-dimensional space. Jackknifed test is a leave one out method (POWER & WARE, 1986), recognizing one sample as unknown, and its reliability is really high.

Comparing with discriminant analysis, SIMCA is rather a newer method. SIMCA constructs hyperboxes in a hyperspace by utilizing principal component analysis as shown in Fig. 5. Hyperboxes become comprehensible as subvolumes in a surrounding space of higher number of dimensions. Consequently Fig. 5. shows a three-dimensional case of principal components transformation derived from the original, high-dimensional set of observations. The soft

modelling method can even be used if not all samples are assigned to groups in advance. SIMCA allows the presence of "the other samples" or outliers (VOGT, 1987).

So many papers have compared the discriminatory capability in sensory evaluation with that in instrumental analysis. Most of those papers suggested better discrimination obtained from instrumental data than from sensory evaluation (LELAND et al., 1987; LINDSAY, 1977). Discriminative sensory evaluation for quality control can be replaced by instrumental analysis in not far future, while affective sensory test will remain.

5.3. Extraction of information from data matrix

Information contained in many predictor variables is extracted and contracted into smaller number of hypothetical variables by using principal component analysis (PCA) or factor analysis (FA). In geometrical relationship, every principal component (PC) intersects orthogonally (Fig. 6), i.e., no correlation existing among PCs. The first PC rounds up the most abundant information in it. The second PC contains secondary abundant information but it should be orthogonal against the first PC.

The contraction of data to find out the latent key factors and to examine the sample distribution based on the calculated factor scores or principal component scores may be the purpose of the application of PCA and FA in

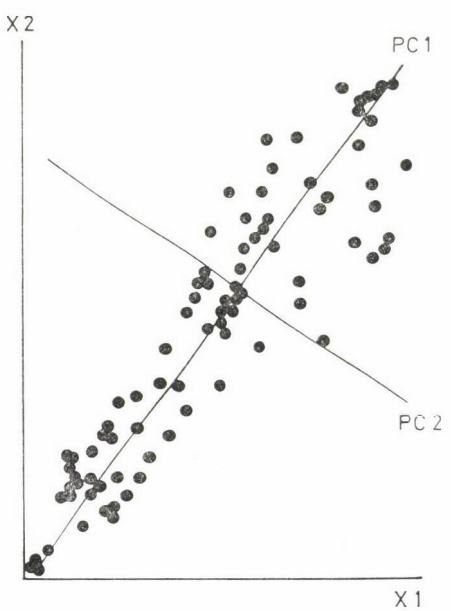


Fig. 6. Principal component analysis of a two dimensional data set

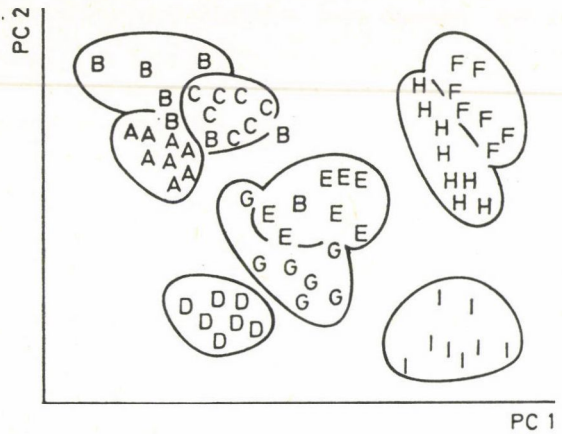


Fig. 7. Plot of soy sauce samples on the first and second PC scores calculated from GC data

flavour analysis (AISHIMA et al., 1979; JACOBSON et al., 1979; GAYDOU et al., 1984). Finding the latent factors in the data matrix is the principal aim in FA. Various algorithms for rotation of factor axes have been created in order to interprete meanings implied in latent factors (DILLON & GOLDSTEIN, 1984). Extracted principal components or factors have advantage over the original

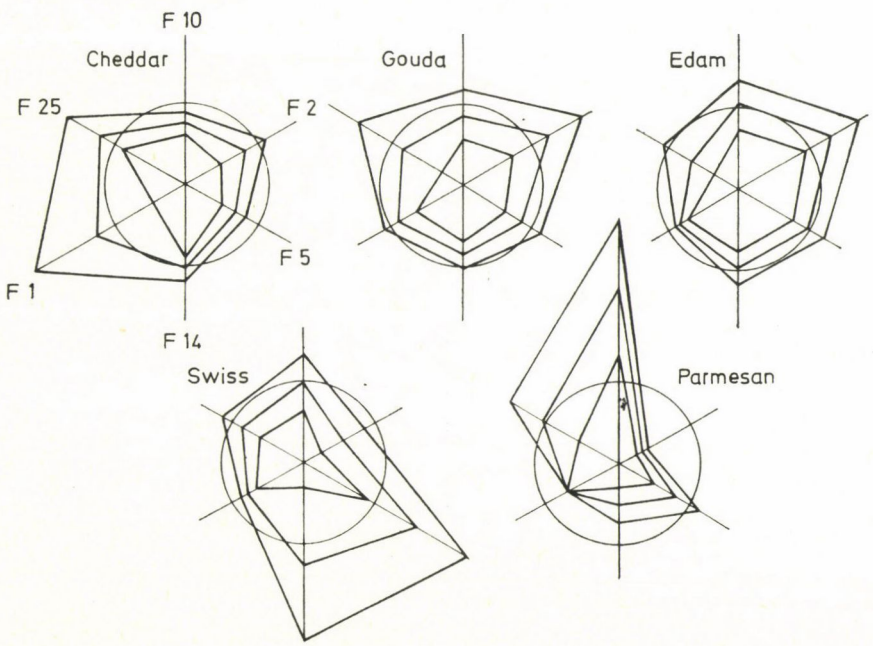


Fig. 8. Factor patterns of five cheese varieties. Six factors were selected by stepwise LDA. Factors were extracted from a matrix integrating GC and HPLC data

variables due to the orthogonality and lesser number. Other multivariate analyses such as MRA, LDA or cluster analysis can be therefore efficiently applied to PCs or FAs (AISHIMA, 1979a; AISHIMA et al., 1979b). Regression analysis on PCs are widely used because the problems caused by multicollinearity are avoided in this case. Programs combining PCA and MRA are available, e.g., BMDP4M (DIXON, 1985).

Soy sauce samples were plotted on the first and second PC scores (Fig. 7) (AISHIMA, 1979b). Samples belonging to the brands A, C, D, I, E & G, or H & F make well separated clusters; discrimination within E & G and H & F appears arbitrarily. On the other hand samples of brand B do not make their own cluster due to the lack of uniformity in their flavour quality. Thus unsupervised pattern recognition was carried out. Flavour profiles of five different cheese varieties are shown in Fig. 8 (AISHIMA et al., 1986). Discriminant analysis was carried out for the FAs which were extracted from combined GC and HPLC data. Factor patterns were generated using the six factors which contribute discriminating cheese varieties.

5.4. Sample clustering

Recently, application number of cluster analysis is increasing in chemometrics area (MASSART & KAUFMAN, 1983; ROMESBERG, 1984). Many different algorithms are available to quantify the similarity or distances among samples

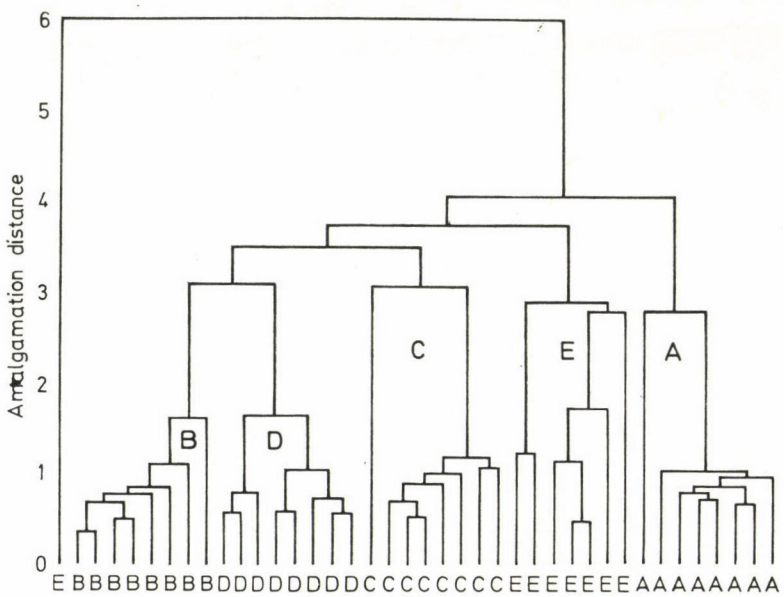


Fig. 9. Clustering of Worcestershire sauce samples. A, B, C, D and E stand for brands

in cluster analysis (JACOBSON & GUNDERSON, 1986). Cluster analysis is a typical unsupervised method. Consequently there are no statistical tests available for the results of cluster analysis. The appropriateness of the output dendrogram from cluster analysis should be evaluated on the basis of our experiences or subjectiveness. Utilization of cluster analysis is classified into two different categories. Firstly, if no information about the data set is obtained, cluster analysis is applied before employing other statistical analysis to constructing hypothesis for the data structure. Secondly, if the results from other multivariate analysis showed certain tendency, then cluster analysis is applied to confirm the results. Regarding quality control, we can, detect outliers in a sample set by cluster analysis as shown in Fig. 9 (AISHIMA 1986). According to the author's experience, using too many variables disturbs the construction of ordered cluster. It may be due to lowering the S/N (signal to noise) ratio, in other words increase in noise term.

6. Simplex optimization

6.1. Simplex optimization

Optimization is one of the major elements in chemometrics. We can apply ordinary one-at-a-time optimization only in the system where interaction among factors does not exist (SAGUY et al., 1984), when we try to optimize system parameters. Experimental design such as factorial design

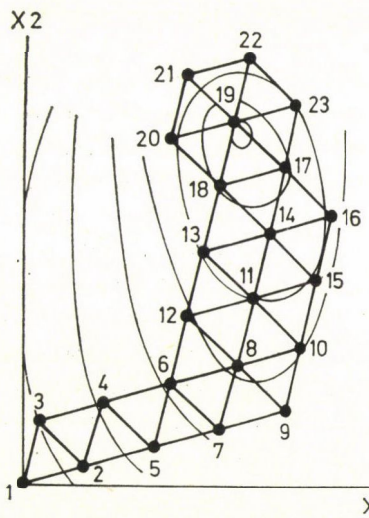


Fig. 10. Movement of regular simplexes over a response surface

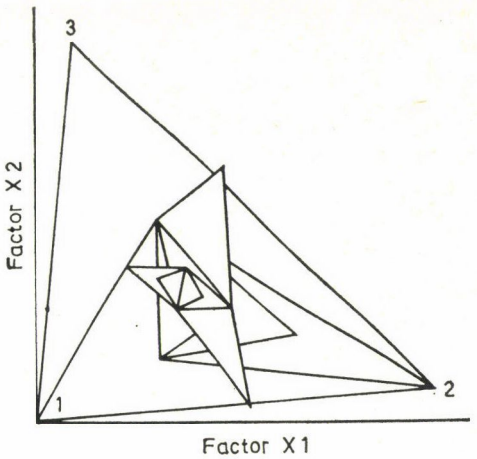


Fig. 11. Movement of simplexes in a modified simplex method

or fractional factorial design can be applied to optimization of such a system in which factors are correlating each other. But large number of experiments are needed in these methods. Response surface methodology (RSM) has been used for such cases. A premise of RSM is the existence of a smooth unimodal response surface which should also be quadratic around optimum area (VUATAZ, 1986; BOX & DRAPER, 1987), however, no one can guarantee the smoothness. Simplex optimization is very useful for such cases (BURTON & NICKLESS, 1987) because simplex optimization does not assume any specific response functions which are inevitable in RSM. Further, location of optimum is only calculated in RSM, while optimum conditions can be pinpointed by simplex optimization through smaller number of experiments.

A simplex is a geometrical figure which has one more vertices than the number of variables or dimensionality, triangles in two factor cases and tetrahedrons in three factor cases. Triangles move over a response surface towards optimum in the regular simplex method by SPENDLEY and co-workers (1962) (Fig. 10). Disadvantages of the regular simplex method in which simplex size was fixed was overcome by the modified simplex methods (MSM) (NELDER & MEAD, 1965; DEMING & MORGAN, 1973) or super-modified simplex method (SMS) (ROUTH et al., 1977), in which simplex size is expanded to accelerate approaching speed towards optimum and is contracted to finely adjust location when simplex has already approached to the vicinity of optimum (Fig. 11). Further improvement in efficiency of optimization was reported by incorporating mapping to visualize response surface (NAKAI et al., 1984; AISHIMA & NAKAI, 1986).

6.2. Flavour optimization

Application of simplex optimization so far has mainly been focused on searching the best conditions for instrumental operation, especially in HPLC analysis (BERRIDGE, 1986; SCHOENMAKERS, 1986). However, simplex optimization is a very versatile method even for food production if we can define the response and factors in a system.

Table 1

Optimization of juice formulation by MSM

Vertex no.	Essence No.			Response S(A,B)
	1 (0.00—0.15)	2 (0.00—0.10)	3 (0.00—0.05)	
1 (Initial simplex)	0.000	0.000	0.000	0.680
2 (Initial simplex)	0.141	0.024	0.012	0.765
3 (Initial simplex)	0.035	0.094	0.012	0.765
4 (Initial simplex)	0.035	0.024	0.047	0.770
5 (Reflection)	0.141	0.094	0.047	0.725
6 (Contraction)	0.106	0.071	0.035	0.752
7 (Reflection)	0.035	0.024	0.012	0.765
19 (Reflection)	0.070	0.053	0.000	0.779
41 (Reflection)	0.076	0.046	0.001	0.779
42 (Contraction)	0.071	0.051	0.001	0.779

Boundary ratio of essence to the concentrate.
Pattern similarity: $1.00 \geq S(A, B) \geq 0.00$

Simplex optimization was applied to flavour simulation of fresh strawberry juice by finding the optimum blending ratio of essences to the juice concentrate (AISHIMA et al., 1987). The optimum blending ratio was searched by two different ways. Firstly, quantitative GC data of fresh juice, essences and concentrate were input into a computer (IBM PC). And then simplex algorithm started to find out the best blending ratio of essences to the concentrate consecutively by calculating pattern similarity between the GC patterns of simulated juice and fresh one (Table 1). Secondly, the optimum blending ratio was searched by doing experiments composed of juice blending, aroma extraction using SDE method, GC analysis and calculation of pattern similarity. The highest pattern similarity was attained at the 24th experiment. Final sensory test confirmed that there was no significant difference among the flavour of fresh juice and differently simulated juices. Concerning optimization of gel viscosity, a successful result was reported on finding the best ratio of calcium, orthophosphates and pyrophosphates to milk casein by fixing the heating temperature and time (NAKAI et al., 1984). A Brookfield viscometer was applied to measuring the response in the optimization.

Understanding the theory of simplex optimization does not require any backgrounds of sophisticated mathematics or statistics. The author can say that simplex optimization will propagate among food scientists and flavour researchers as well as food technologists in near future.

Literature

AISHIMA, T. (1979a): Classification of soy sauce on principal components in GC profiles. *Agric. biol. Chem., 43,* 1905–1910.

AISHIMA, T. (1979b): Evaluation and discrimination of soy sauce by computer analysis of volatile profiles. *Agric. biol. Chem., 43,* 1711–1718.

AISHIMA, T. (1981): Glass capillary chromatographic profiles of soy sauce aroma correlated with sensory assessment. *Agric. biol. Chem., 45,* 2847–2853.

AISHIMA, T. (1983): Relationships between gas-chromatographic profiles of soy sauce volatiles and organoleptic characteristics based on multivariate analysis. — in: CHARALAMBOUS, G. & INGLETT, G. *Instrumental analysis of foods.* Academic Press, New York, pp. 37–56.

AISHIMA, T. (1986): Application of multivariate analysis to silica capillary GC profiles to differentiate the aroma characteristics of Worcestershire sauces. — in: CHARALAMBOUS, G. *The shelf life of foods and beverages.* Elsevier Science Publishers, Amsterdam, pp. 755–774.

AISHIMA, T., MOEHLER SMITH, A. M. & NAKAI, S. (1986): Pattern recognition of integrated GC and HPLC data of cheese components for objective discrimination of five varieties. *Proceedings of the 192nd ACS national meeting.* Anaheim, AGFD 40.

AISHIMA, T., NAGASAWA, M. & FUKUSHIMA, D. (1979): Differentiation of the aroma quality of soy sauce by statistical evaluation of gas-chromatographic profiles. *J. Fd Sci., 44,* 1723–1727, 1731.

AISHIMA, T. & NAKAI, S. (1986): Centroid mapping optimization: a new efficient optimization for food research and processing. *J. Fd Sci., 51,* 1297–1300, 1310.

AISHIMA, T. & NOBUHARA, A. (1977): Flavour evaluation by multiple regression analysis of gas chromatographic patterns in soy sauce. *Fd Chem., 2,* 161–175.

AISHIMA, T., WILSON, D. L. & NAKAI, S. (1987): Application of simplex algorithm to flavour optimization based on pattern similarity of GC profiles. — in: MARTENS, M., DALEN, G. A. & RUSSWURM JR., H. *Flavour science and technology.* John Wiley & Sons, Chichester, pp. 501–508.

AMERINE, M. A., PANGBORN, R. M. & ROESSLER, E. B. (1965): *Principles of sensory evalution of food.* Academic Press, New York and London.

ARNOLD, G. M. & WILLIAMS, A. A. (1986): The use of generalised procrustes techniques in sensory analysis. — in: PIGGOTT, J. R. *Statistical procedures in food research.* Elsevier Applied Science, London, pp. 233–253.

ASTM COMMITTEE E-18 (1981): *Guidelines for the selection and training of sensory panel members, STP 758.* American Chemical Society for Testing and Materials, Philadelphia.

BERRIDGE, J. C. (1986): *Techniques for the automated optimization of HPLC separations.* John Wiley & Sons, Chichester.

BOX, G. E. P. & DRAPER, N. R. (1987): *Empirical model-building and response surface.* John Wiley & Sons, New York.

BREEN, J. J. & ROBINSON, P. E. (1985): *Environmental applications of chemometrics. ACS symposium series 292.* American Chemical Society, Washington, D. C.

BURTON, K. W. C. & NICKLESS, G. (1987): Optimisation via simplex. — Part I. Background, definition and a simple application. *Chemometrics and Intelligent Lab. Systems, 1,* 135–149.

BUTTERY, R. G. (1981): Vegetables and fruit flavours. — in: TERANISHI, R., FLATH, R. A. & SUGISAWA, H.: *Flavour research — recent advances.* Marcel Dekker, New York, pp. 175–216.

DEMING, S. N. (1986): Chemometrics: an overview. *Clin. Chem., 32,* 1702–1706.

DEMING, S. N. & MORGAN, S. L. (1973): Simplex optimization of variables in analytical chemistry. *Analyt. Chem., 45,* 278A–283A.

DEMING, S. N. & MORGAN, S. L. (1983): Teaching the fundamentals of experimental design. *Analytica chim. Acta, 150*, 183–198.

DEMING, S. N. & MORGAN, S. L. (1987) *Experimental design: a chemometric approach.* Elsevier, Amsterdam.

DERDE, M.-P., COOMANS, D. & MASSART, D. L. (1984): SIMCA demonstrated with characterization and classification of Italian olive oil. *J. Ass. off. anal. Chem., 67*, 721–727.

DILLON, W. R. & GOLDSTEIN, M. (1984): *Multivariate analysis-methods and applications.* John Wiley & Sons, Chichester, pp. 53–106.

DIXON, W. J. (1985): *BMDP statistical software.* University of California Press, Berkeley.

DRAVNIEKS, A. (1974): Approaches to subjective/objective correlations in flavor. — in: POWERS, J. J. & MOSKOWITZ, H. R. : *Correlating sensory objective measurements, ASTM special technical publication 594.* American Society for Testing and Materials, Philadelphia, pp. 5–35.

DRUSHEL, H. V. (1983): Needs of the chromatographic-detectors. *J. Chromat. Sci., 21*, 375–384.

FISHER, B. V. & JONES, R. (1987): Chemometrics in pharmaceutical analysis. *J. pharm. biomed. Anal., 5*, 455–467.

FLATH, R. A. & FORREY, R. R. (1977): Volatile components of papaya. *J. agric. Fd Chem., 25*, 104–109

FORINA, M., ARMANINO, C., CASTINO, M. & UBIGLI, M. (1986): Multivariate data analysis as a discriminating method of the origin of wines. *Vitis, 25*, 189–201.

FORINA, M., LANTERI, S. & ARMANINO, C. (1987): Chemometrics in Food chemistry. *Topics Curr. Chem., 141*, 93–143.

GAYDOU, E. M., IATARIDES, M. C., RAMANANARIVO, R., ARTAUD, J. & PEIFFER, G. (1984): Pattern recognition analysis of fatty acids. Application to beef fat tissue classification. *J. agric. Fd Chem., 32*, 651–655.

HAITOVSKY, Y. (1987): On multivariate ridge regression. *Biometrika, 74*, 563–570.

HALL, R. L. (1968): Food flavors. Benefits & problems. *Fd Technol., 22*, 1388–1392.

HERRES, W. (1984): Capillary GC-FTIR analysis of volatiles: HRGC-FTIR. — in: SCHREIER, P. *Analysis of volatiles.* Walter de Gruyter, Berlin, pp. 183–217.

JACOBSON, T. & GUNDERSON, R. W. (1986): Applied cluster analysis. — in: PIGGOTT, J. R. *Statistical procedures in food research.* Elsevier Applied Science, London, pp. 361–408.

JACOBSON, T., VOLDEN, R., ENGAN, S. & AUBERT, O. (1979): A chemometric study of some beer flavour components. *J. Inst. Brew., 85*, 265–270.

JONES, L. V., RERYAM, D. R. & THURSTONE, L. L. (1955): Development of a scale for measuring soldiers' food preference. *Fd Res., 20*, 512–520.

KOWALSKI, B. R. (1977): *Chemometrics: theory and applications, ACS symposium series No. 52.* American Chemical Society, Washington, D. C.

KOWALSKI, B. R. (1981): Analytical chemistry as an information science. *Trends analyt. Chem., 1*, 71–74.

KRUKONIS, V. J. (1985): Supercritical fluid extraction in flavor application. — in: BILLS, D. D. & MUSSINAN, C. J. *Characterization and measurement of flavor compounds. ACS symposium series No. 289.* American Chemical Society, Washington, D. C., pp. 154–175.

LARRECHI, M. S., GUASCH, J., RIUS, F. X. & SOLE, A. (1987): Characterization of Catalan red wines by pattern recognition methods. *Vitis, 26*, 172–182.

LELAND, J. V., LAHIFF, M. & REINECCIUS, G. A. (1987): Predicting intensities of milk off-flavors by multivariate analysis of gas-chromatographic data. — in: MARTENS, M., DALEN, G. A. & RUSSWURM, JR. H. *Flavour science and technology.* John Wiley & Sons, Chichester, pp. 453–467.

LINDSAY, R. C. (1977): Objective measurements of the flavor quality of beer. — in: SCANLAN, R. A. *Flavor quality: objective measurement. ACS symposium series, No. 51.* American Chemical Society, Washington, D. C., pp. 89–103.

MARTENS, H. (1985): *Multivariate calibration: quantitative interpretation of non-selective chemical data.* The Norwegian Institute of Technology, University of Trondheim, Trondheim.

MARTENS, H. & RUSSWURM JR., H. (1983): *Food research and data analysis.* Applied Science Publishers, London.

MARTENS, M., DALEN, G. A. & RUSSWURM JR., H. (1987): *Flavour science and technology.* John Wiley & Sons, Chichester.

MARTENS, M. & HARRIES, J. M. (1983): A bibliography of multivariate statistical methods in food science and technology. — in: MARTENS, H. & RUSSWURM, JR., H. *Food research and data analysis*. Applied Science Publishers, London, pp. 493–518.

MARTENS, M. & MARTENS, H. (1986): Partial least squares regression. — in: PIGGOTT, J. R.: *Statistical procedures in food research*. Elsevier Applied Science, London, pp. 293–359.

MASSART, D. L. & KAUFMAN, L. (1983): *The interpretation of analytical chemical data by use of cluster analysis*. John Wiley & Sons, New York.

MASSART, D. L., VANDEGINSTE, B. G. M., DEMING, S. N., MICHOTTE, Y. & KAUFMAN, L. (1987): *Chemometrics: a textbook*. Elsevier Science Publishers, Amsterdam.

MECREDY, J. M., SONNEMANN, J. C. & LEHMANN, S. J. (1974): Sensory profiling of beer by a modified QDA method. *Fd Technol., 28*, (11), 36–41.

MOSKOWITZ, H. R. & CHANDLER, J. W. (1977): New uses of magnitude estimation. — in: BIRCH, G. G., BRENNAN, J. G. & PARKER, K. J. *Sensory properties of foods*. Applied Science Publishers, London, pp. 189–211.

NAKAI, S., KOIDE, K. & EUGSTER, K. (1984): A new mapping super-simplex optimization for food product and process development. *J. Fd Sci., 49*, 1143–1148, 1170.

NELDER, J. A. & MEAD, R. A. (1965): A simplex method for function minimization. *Comput. J., 7*, 308–313.

NORUSIS, M. J. (1985): *SPSS advanced statistics guide*. McGraw-Hill Book Company, New York.

NUMEZ, A. J. & GONZALES, L. F. (1984): Pre-concentration of headspace volatiles for trace organic analysis by gas-chromatography. *J. Chromat., 300*, 127–162.

PANGBORN, R. M. V. (1984): Sensory techniques of food analysis. — in: GRUENWEDEL, D. W. & WHITAKER, J. R. *Food analysis principles & techniques*. Vol. 1. *Physical characterization*. Marcel Dekker, New York, pp. 37–93.

PIGGOTT, J. R. (1986): *Statistical procedures in food research*. Elsevier Applied Science, London.

POWERS, J. J. (1982): Techniques of analysis of flavours: integration of sensory and instrumental methods. — in: MORTON, I. D., MACLEOD, A. J. *Food flavours. - Part A. Introduction*. Elsevier Scientific Publishing Company, Amsterdam, pp. 121–168.

POWERS, J. J. & KEITH, E. S. (1968): *Stepwise discriminant analysis of gas-chromatographic data as an aid in classifying the flavor quality of foods. J. Fd. Sci., 33*, 207–218.

POWERS, J. J. & WARE, G. O. (1986): Discriminant analysis. — in: PIGGOTT, J. R. *Statistical procedures in food* research. Elsevier Applied Science, London. pp. 125–180.

RAY, A. A. (1985): *SAS user's guide: statistics*. SAS Institute, Carey, 1985.

RAMOS, L. S., BEEBE, K. R., CAREY, W. P., SANCHEZ, M. E., ERICKSON, B. C., WILSON, B. E., WANGEN, L. E. KOWALSKI, B. R. Chemometrics. Analyt. Chem., 58, 294R–315R.

ROMESBERG, H. C. (1984): *Cluster analysis for researchers*. Lifetime Learning Publication, Belmonte.

ROUTH, M. W., SWARTZ, P. A. & DENTON, M. B. (1977): Performance of the super modified simplex. *Analyt. Chem., 49*, 1422–1428.

SAGUY, I., MISHKIN, M. A. & KAREL, M. (1984): Optimization methods and available software. Part I. *CRC crit. Rev. Fd Sci. Nutr., 20*, 275–299.

SCHOENMAKERS, P. J. (1986): *Optimization of chromatographic selectivity — a guide to method development*. Elsevier, Amsterdam.

SENSORY EVALUATION DIVISION OF IFT (1981): Sensory evaluation guide for testing food and beverage products. *Fd Technol., 35*, (11), 50–59.

SHIFFMAN, S. S., REYNOLDS, M. L. & YOUNG, F. W. (1981): *Introduction to multidimensional scaling: theory methods and applications*. Academic Press, New York.

SIMON, P. W., PETERSEN, C. E. & LINDSAY, R. C. (1980): Correlation between sensory and objective parameters of carrot flavor. *J. agric. Fd Chem., 28*, 559–562.

SPENDLEY, W., HEXT, G. R. & HIMSWORTH, F. R. (1962): Sequential application of simplex designs in optimisation and evolutionary operation. *Technometrics, 4*, 441–461.

STANLEY, D. W., VOISEY, P. W. & SWATLAND, H. J. (1980): Texture-structure relationships in bacon lean. *J. Texture Stud., 11*, 217–238.

STEVENS, S. S. (1946): On the theory of scales of measurement. *Science, 103*, 677–680.

STONE, H., SIDEL, J., OLIVER, S., WOOLSEY, A. & SINGLETON, R. C. (1974): Sensory evaluation by quantitative descriptive analysis. *Fd Technol., 28*, (11), 24–34.

SZCZENIAK, A. S. (1987): Correlating sensory with instrumental texture measurements — an overview of recent developments. *J. Texture Stud., 18*, 1–15.
TERANISHI, R., FLATH, R. A. & SUISAWA, H. (1981): *Flavor research—recent advances.* Marcel Dekker, New York.
VARMUZA, K. (1980): *Pattern recognition in chemistry.* Springer-Verlag, Berlin, pp. 62–71.
VOGT, N. B. (1987): Soft modeling and chemometrics. *Chemometrics and Intelligent Lab. Systems, 1*, 213–231.
VUATEZ, L. (1986): Response surface methods. — in: PIGGOTT, J. R. *Statistical procedures in food research.* Elsevier Applied Science, London, pp. 101–123.
WOODRUFF, H. B. (1984): Expert systems. — Part II. *Analyt. Chem., 56*, 1314A–1332A.

BOOK REVIEWS

Legumes

Chemistry, Technology and Human Nutrition

Ruth H. MATTHEWS (Ed.)

Marcel Dekker, Inc., New York and Basel, 1989; 408 pages

The book gives an accurate and comprehensive information on chemistry and technology of the production, processing and properties of various legumes and legume products.

Each topic is presented comprehensively by an authoritative source. Chemistry and technology of refined oils, protein isolates and concentrates, fermentation products are described. The complex subject of legume nutrition is covered in chapters on nutrient composition, bioavailability of nutrients and antinutritional factors. Nutrient composition data are given and discussed for such foods as cooked and canned legumes, sprouted legumes and other products as tofu, tempeh, miso and natto. Animal feeds are also covered.

The book contains 10 chapters: Culture and genetics of grain legumes (6 parts); Harvesting and storage of legumes (5 parts); Refined oils (4 parts); Isolated soy proteins (6 parts); Legumes protein flour and concentrates (3 parts); Fermented products (9 parts); Nutrient composition of raw, cooked, canned and sprouted legumes (4 parts); Nutrient content of other legume products (6 parts); Animal feed uses of legume (10 parts); Antinutritional factors (10 parts). Each chapter has a reference which gives a good source for research works.

The book is advisable for food scientists, technologists, nutritionists and for students in nutrition and food science.

I. VARSÁNYI

A classification of food and physical properties

R. JOWITT

Food Science Publishers Ltd, England, 1989; 93 pages

The publisher has dealt with a special task when "A classification of foods and physical properties" was issued. The classification published in one line on four languages — in English, German, French and Spanish — may be well used for all working on the field of food science and technology whose mother tongue is not English.

The classification contains 12 food groups, several of them represent a food industrial branch (e.g. dairy products, bakery products, alcoholic beverages). This raw material centric grouping is supplemented by the Additives; Water, ice; Treatment classification; and Physical property classification chapters.

Obviously, one may ask why this classification was made and why were others left out. A clear explanation is given to this in the introduction and the system is not closed and may be extended indefinitely from any chosen point by further subclassification to suit individual needs. I fully agree with Prof. Jowitt who stated that it is not only possible to continue this work but it should be extended, supplemented and further developed by the latest results achieved on the field of food science and technology.

The book is an important tool for not only specialists working in research and industrial management but also for high school and university students, too.

I. VARSÁNYI

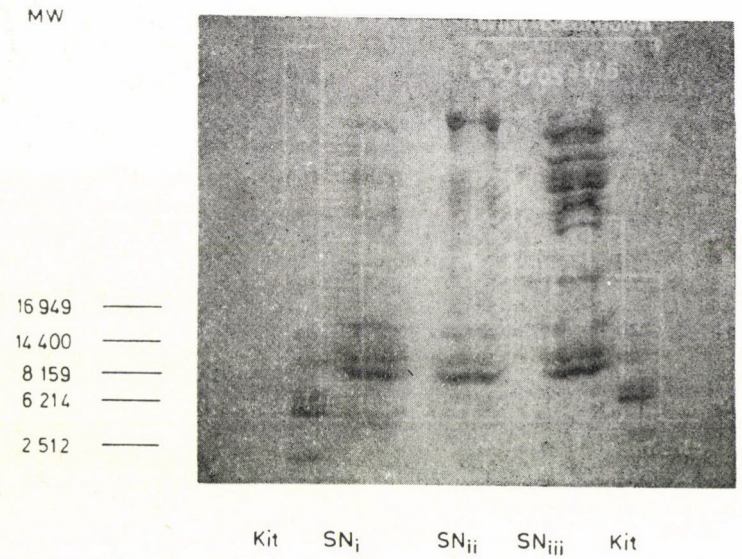
ERRATUM

ISOLATION, PURIFICATION AND DETERMINATION OF LOW MOLECULAR WEIGHT PEPTIDES FROM BREAD DOUGH

C. Benedito de Barber, J. A. Prieto, C. Collar and S. Barber

Volume 18, No. 1. p. 59, Fig. 3.

The right positon of the photo:



A

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

RECENTLY ACCEPTED PAPERS

Colour and carotenoid content of quick-frozen tomato cubes during frozen storage
URBÁNYI, Gy. & HORTI, K.

A comparative study of three procedures for the isolation of volatile flavour compounds from a model solution
GAGNON, M. J. & MARCOUX, A. M.

Dietary olive oil and liver cholesterol in rats
BEYNEN, A. C.

Reduction of panel variances by a simple two-step normalization procedure for graphical line scale
WEISS, J. & ZENZ, H.

Designed protein modification by enzymatic technique
HAJÓS, Gy., HALÁSZ, A. & BÉKÉS, F.

NOTICE TO CONTRIBUTORS

General. Manuscripts in English or Hungarian should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

Title. The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

Summary. The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

Text. The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

Symbols. Units, symbols and abbreviations should be used according to SI (System International) rules. Unusual abbreviations must be explained in the text or in an appendix.

Figures and tables. Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

References. The reference list (Literature) should be in alphabetical order as follows:

Periodicals: Names and initials of all the authors; year of publication in parentheses; colon; title of the paper; title of the periodical; inclusive page numbers.

Books: Names and initials of all the authors; the year of publication in parentheses; colon; title of the book; publishing firm, place of publication; inclusive page numbers.

Detailed instructions for publishing in Acta Alimentaria are available from the Editorial Office.

Authors will receive one set of proofs which must be corrected and returned at the earliest convenience to the Editorial Office. In this phase major alterations of the text cannot be accepted.

Offprints. We supply 100 offprints free of charge. Additional copies can be ordered.

No page charges are levied on authors or their institutions.

Acta Alimentaria is surveyed by Current Contents/Agriculture, Biology and Environmental Sciences, ASCA, BIOSIS, Nutrition Abstracts and Reviews.

ACTA ALIMENTARIA

VOLUME 18 No. 2 — 1989

CONTENTS

Index: 23.069

ACTA ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 18 **September 1989** **Number 3**

Akadémiai Kiadó
Budapest

ISSN 0139—3006

CODEN ACALDI

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

Distributor:

KULTURA, Hungarian Foreign Trading Company
P.O. Box 149, H-1389 Budapest 62, Hungary

Publication programme, 1989: Volume 18 (4 issues)
Subscription prices per volume: US $ 130,00 plus 15% postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 18

1989

AKADÉMIAI KIADÓ
BUDAPEST

Acta Alimentaria, Vol. 18 (3), pp. 247—267 (1989)

COLOUR AND CAROTENOID CONTENT OF QUICK-FROZEN TOMATO CUBES DURING FROZEN STORAGE

GY. URBÁNYI and K. HORTI

Department of Food and Refrigerating Technology,
Institute of Food Technology, University of Horticulture
and Food Industry, H-1118 Budapest, Ménesi út 45. Hungary

(Received: 21 March 1988; accepted: 27 September 1988)

One of the most important quality characteristics of tomato is its colour. Thus, it was found important to investigate the behaviour during frozen storage of the colour and pigment content in quick-frozen tomato cubes, manufactured in increasing amounts in recent years. In a part of the experiments the test material was gained from the same tomato cultivar at different stages of maturity, while in the other part the fully ripe fruits of three different cultivars were used. The colour of tomatoes, frozen at —30 °C and stored at —20 °C, was studied by the tristimulus method and the amount of carotenoids by photometry after separation by column chromatography.

The colour of the tomato products was found to change continuously during frozen storage. The process appeared in the diminishing of the red character and increasing of the yellow character and simultaneous lightening of the colour. There was no characteristic change in saturation. The extent of change was maturity-dependent.

A linear correlation was observed between colour and time of storage. In the case of values b*, L* and TCI the correlation was found to be close and significant.

The quantity of carotenoids diminished during frozen storage. The reduction of the quantity of pigments present in the tomato in the largest amount, as well as that of all the carotenoids is in close linear correlation with the storage period.

The quantity of the same pigments is in close linear correlation with b*, L* colour characters and the Tomato Colour Index (TCI). Thus, the relatively more rapidly determinable colour characters permit to draw conclusions not only as to the change of colour but to the quantitative change of pigments, too.

Keywords; colour of tomato, carotenoids of tomato, colour stimulus

Colour is one of the most important quality characteristics of tomatoes. The consumer associates to the deeper red colour a more mature raw material as well as a better flavour. Therefore, research into the colour of tomatoes has a past of several decades. Gaylord has published several papers on this subject around the 1920s (FRANCIS & CLYDESDALE, 1975). Since the colour is a very important factor of judging the raw material it has been thoroughly investigated. Many papers have been published in relation to the colour of tomato puree, as well.

In recent years the preservation of tomato by freezing came into prominence. Tomato frozen in cubes became a very popular product. Since the adequate colour is very important in relation to this product, too, the question

whether the colour of the cubes changes during frozen storage, was raised. Simultaneously quantitative changes in the main carotenoids responsible for the colour of tomato were also investigated.

YEATMAN (1969; 1976) among many other authors investigated in details the problems related to the colour of tomato and tomato products.

Problems related to measuring tomato colour were thoroughly dealt with in Hungary, too. BONTOVITS (1960; 1974) studied the possibility of defining the colour stimulus of raw tomatoes and of tomato puree objectively and by the minimum of parameters. He suggested the quotient x/y and the function xy/Y in the CIE xyY system. In another of his papers he gave a general overview on the problems of colour determination and on the applicable systems particularly in view of tomato puree production (BONTOVITS, 1980; HARKAY & BONTOVITS, 1981). In studying the effects of technological processes (BONTOVITS, 1979) he subjected to investigation the parameters used for the characterization of tomato colour.

American researchers studied the colour of tomatoes during ripening and developed procedures (WORTHINGTON, 1971; WORTHINGTON et al., 1973; NATTUVETTY & CHEN, 1980; DIXON & HOBSON, 1984).

The effect of temperature upon the formation of colour was studied by HALL (1961) and KOSKITALO and ORMROD (1972).

A number of authors examined the development of carotenoids responsible for the colour of tomato and the composition of carotenoids in tomato (THOMPSON, 1961; TOMES, 1963; THOMPSON et al., 1965; THOMPSON et al., 1967; JEN, 1974; RAYMUNDO et al., 1976). Several of them studied the effect of temperature, too.

Not many publications were found pertinent to the influence of freezing and frozen storage on the colour of tomato. Only a slight change was observed by DRDÁK and SORMAN (1979) in tomato puree kept in storage at -15 °C for 9 months.

BONTOVITS (1960) studied the correlation of lycopene content with values x/y and xy/Y as suggested by himself, in tomato puree. He found a close correlation ($r = 0.753$) between the lycopene content and x/y quotient. The correlation to the lightness factor (Y) was much less close being $r = 0.610$.

The correlation between colour and pigment content at various stages of ripening was studied by WATADA and co-workers (1976). Close correlation was found between the difference in absorbances as measured at 710 and 780 nm and the chlorophyll content as well as between the difference in absorbance as measured at 570 and 780 nm and the pigment content. For lycopene the value of r was 0.97 and the regression equation:

$$A(570\text{–}780 \text{ nm}) = 0.604 + 0.0431 \text{ lycopene.}$$

For beta-carotene: $r = 0.95$, $A(550\text{–}580 \text{ nm}) = -0.433 + 0.297$ beta-carotene.

KOSKITALO and ORMROD (1972) investigated the effect of beta-carotene

and lycopene content on the extent in the fruit of turning red and they established the pigment content at which the fruit appears red.

NOBLE (1975) found close correlation between the a_L value and the total pigment content ($r = 0.978$) and the lycopene content ($r = 0.907$), respectively.

DRDAK and co-workers (1977) measured the colour of tomato puree with Momcolor tristimulus colorimeter and studied the correlation between the colour coordinates (x, y) and the total carotenoid, lycopene and beta-carotene content. In every case the correlation was satisfactory and the coefficients varied between 0.752 and 0.945. Similar investigations related to frozen products were not found in the literature.

1. Materials and methods

1.1. Materials

In the first year (1985/86) the cultivar Kecskeméti Jubileum (Jubilee of Kecskemét) was used in the experiments, field grown under garden conditions, at three different stages of ripeness. The degree of maturity was determined visually as follows:

1st degree of ripeness: skin orange with some yellow spots;

2nd degree of ripeness: cca 60% of the skin red, the rest orange coloured;

3rd degree of ripeness: completely ripe, dark red.

In the season 1986/87 the investigations were carried out on fruit fully mature. The samples were obtained from the Development Enterprise of the Vegetable Research Institute, Kecskemét. The cultivars were the following: Kecskeméti 407 (hereinafter: K1), Kecskeméti Jubileum (K2), Kecskeméti 555 (K3).

The raw material was processed identically in both years. The fruit was processed on the day following harvest. Subsequent to selecting and washing the fruits were diced into cubes of 10 mm edge length and placed in polyethylene bags, then frozen in Lehel-Tyler freezing chamber at −30 °C. When frozen the bags were removed to chambers of −20 °C.

1.2. Methods

1.2.1. Measurement of colour. The colour of the samples was objectively measured with the Momcolor-D tristimulus colorimeter by reflection technique. The white enamel standard of the National Office of Measures, Hungary, No. 80-26-00 was used for comparison. The colour stimulus components of the standard are:

$$X_1 = 64.90,\ X_2 = 15.82,\ Y = 82.91,\ Z = 95.14.$$

Geometry of the measuring head: 0°/45°, optic angle 2°, CIE C illumination. Diameter of the diaphragm 10 mm.

Before experiment the frozen cubes were defrosted in water-bath. A 150 g sample packed in polyethylene pouch was dipped in water-bath of 40 °C for 30 min then pulped in a kitchen-homogenizer. The air beaten in the puree was removed under vacuum and the puree was filled into glass cuvette.

From each material 3 samples were taken and 5 measurements made.

1.2.2. Determination of carotenoids. The method of TOMES (1963) was applied. The pigments were extracted with the mixture of acetone and hexane. After clarification the solution was applied to a column of magnesiumoxide–-diatomaceous earth. The carotenoids eluted from the column in hexane were determined photometrically. A Spectromom 195 d spectrophotometer (Hungary) was used. The result received are tabulated in Tables 4, 5, 6. The average values ($\bar{x}$) and standard deviation ($\pm$ s) were calculated by 3 parallel measurements. Results were related to the dry matter content.

1.2.3. Determination of total dry matter content. The samples were dried in an oven of 105 °C temperature.

These determinations were carried out to relate the carotenoid content to the dry matter content. Thus, these results are not published.

1.2.4. Determination of pH. In the first series of experiments the pH was measured with a Universal Type OP-204/1 (Radelkisz, Hungary) instrument in order to establish whether during storage occurs a change of pH influencing the colour of the sample. Since such change was not observed it did not seem necessary to continue the measurement of pH in the second year. These measurements were carried out only for the sake of control the results thus they are not published.

The samples were tested in the first series of experiments directly after freezing and after about 6 months frozen storage about 7 times. The second series of experiments were tested directly upon freezing and then during 1 year of frozen storage on 9 occasions.

2. Results

2.1. Change in colour as a function of storage time

The colour stimulus characteristics of tomatoes frozen at three different stages of maturity were established in the CIELAB system and are presented in Figs. 1, 2 and 3.

During storage the following changes of colour characters were observed: the change of colour in the least ripe sample as established on the 17th day of storage was hardly visible by the naked eye ($\Delta E^*_{ab} = 0.90$). The slight change appeared in the increase of the yellow character ($\Delta b^* = 0.77$) while

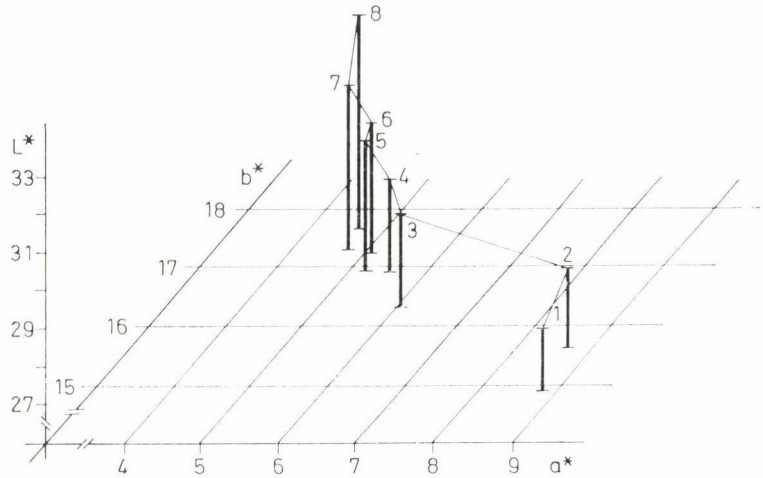


Fig. 1. Colour points in the CIELAB space of colour of a tomato sample at the 1st degree of ripeness. 1. On the day of freezing; 2. on the 17th day; 3. on the 44th day; 4. on the 73rd day; 5. on the 99th day; 6. on the 134th day; 7. on the 155th day; 8. on the 176th day of storage

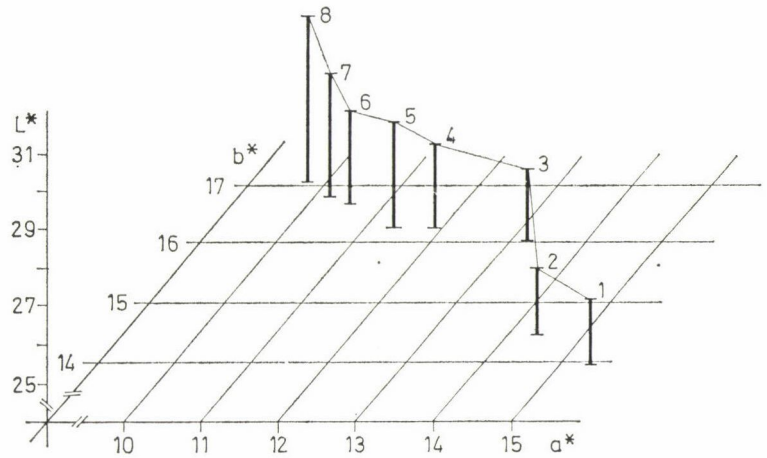


Fig. 2. Colour points in the CIELAB space of colour of a tomato sample at the 2nd degree of ripeness. 1. On the day of freezing; 2. on the 20th day; 3. on the 42nd day; 4. on the 70th day; 5. on the 97th day; 6. on the 132nd day; 7. on the 153rd day; 8. on the 174th day of storage

the red character remained unchanged ($\Delta a^* = 0.16$). During further storage the red character continuously decreased with simultaneous increase in the yellow character (Fig. 1). With the advance of storage time the samples became gradually lighter while the change in saturation was not unambiguous.

The medium ripe sample behaved similarly to the 1st sample (Fig. 2), only already in the 1st phase of storage the change was somewhat greater

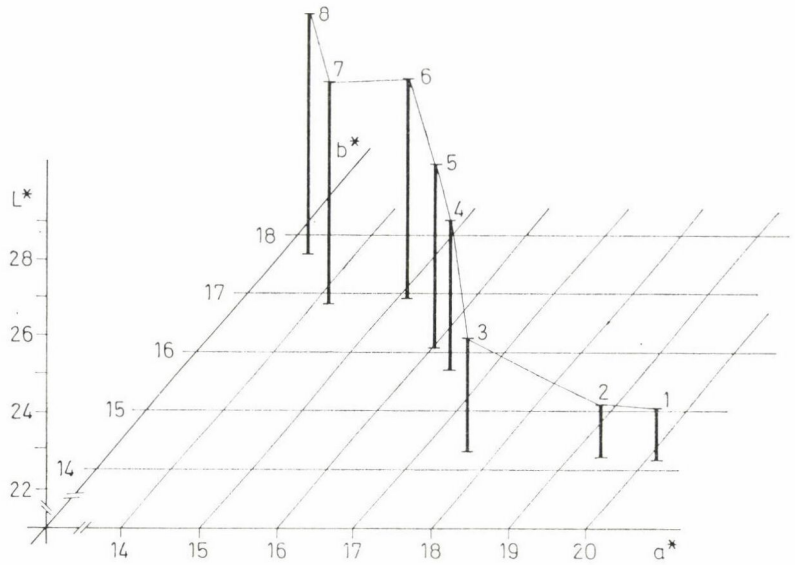


Fig. 3. Colour points in the CIELAB space of colour of a tomato sample at the 3rd degree of ripeness. 1. On the day of freezing; 2. on the 8th day; 3. on the 43rd day; 4. on the 76th day; 5. on the 98th day; 6. on the 133rd day; 7. on the 154th day; 8. on the 175th day of storage

($\Delta E^*_{ab} = 1.16$; $\Delta a^* = 1.07$; $\Delta b^* = 0.46$). Since the 2nd sample was more advanced as regards ripening its colour character was deeper red, less yellow, than that of the less ripe sample. Beside the colour was darker and more saturated.

In the 3rd sample (ripe) the changes occurring during frozen storage were analogous to those occurring in the first two (Fig. 3). In accordance with the higher degree of ripeness the sample retained its deeper red character beside a fainter yellow character, possessed a higher saturation and it was darker, too.

With increasing storage time colour changes became more intense. From the 43rd–76th day onward changes advanced in parallel to the degree of maturity becoming quite considerable by the end of the storage period. The difference in colour of the least ripe sample amounted to ($\Delta E^*_{ab} = 6.33$) on the 176th day. With the medium ripe sample the difference was 6.95 on the 174th day and with the ripest samples it was 9.21 on the 175th day.

It should be noted, however, that the ripest sample, though undergoing the greatest change, was a deeper red at the end of storage than the less ripe samples and also of a higher saturation, as well. There was no significant difference among the samples in the yellow character.

The Tomato Color Index suggested to characterize the colour of tomatoes was also calculated (FRANCIS & CLYDESDALE, 1975). It can be seen that the

colour changes are well characterized by this index, too. During the full storage period the extent of change diminishes with increasing maturity. The ripe sample had a higher TCI value at the end of the storage period than the initially less ripe.

In the second series of experiments the fully ripe fruits of three different tomato cultivars were used. The results of colour measurements are shown in Figs. 4, 5 and 6.

During the one year storage period the changes were similar to those observed in the previous year. The red character diminished in all the three cultivars, while the yellow character increased and the samples became lighter at practically unchanged saturation. Changes were substantially higher in cultivar K2 than in the other two cultivars. After one year the colour difference for the K2 cultivar was $\Delta E^*_{ab} = 13.49$ while for the other two 7.33 and 6.47, respectively. The TCI value decreased from the initial 7.87 to 2.23, while for the other two cultivars from 8.83 to 7.26 and from 8.86 to 7.07, respectively. This was probably due to the fact that the fruits of cultivar K2 were of much looser consistency than those of the other two, therefore a substantial part of the cubes disintegrated and thereby increased the free surface.

Linear regression analysis was carried out to establish the correlation between colour stimulus characters and storage time. The correlation coefficients belonging to the first series of experiments are listed in Table 1, while those of the second series in Table 2.

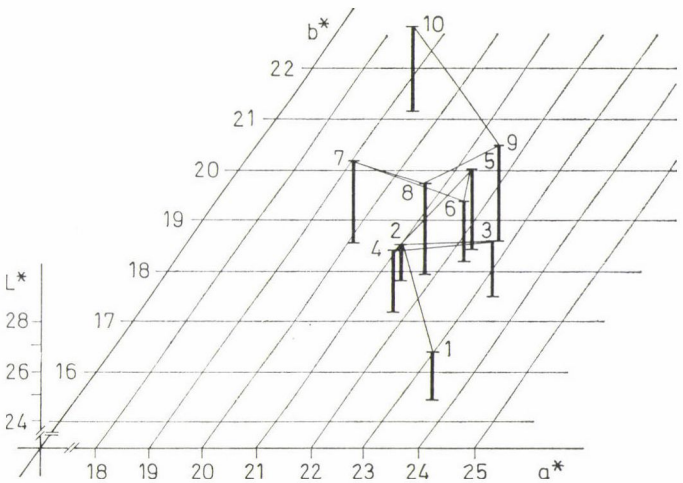


Fig. 4. Colour points in the CIELAB space of colour of the tomato cultivar Kecskeméti 407. 1. On the day of freezing; 2. on the 30th day; 3. on the 72nd day; 4. on the 114th day; 5. on the 154th day; 6. on the 190th day; 7. on the 233rd day; 8. on the 273rd day; 9. on the 296th day; 10. on the 317th day of storage

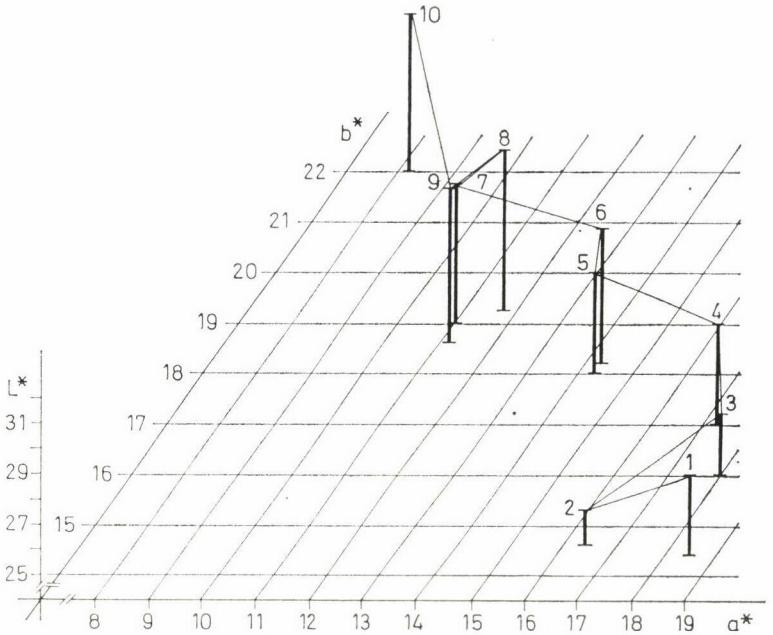


Fig. 5. Colour points in the CIELAB space of colour of the tomato cultivar Kecskeméti Jubileum. 1. On the day of freezing; 2. on the 32nd day; 3. on the 74th day; 4. on the 110th day; 5. on the 152nd day; 6. on the 195th day; 7. on the 228th day; 8. on the 273rd day; 9. on the 298th day; 10. on the 370th day of storage

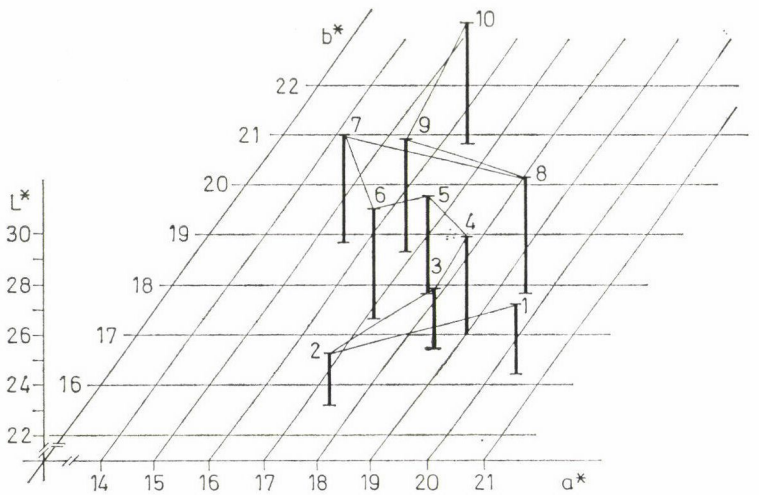


Fig. 6. Colour points in the CIELAB space of colour of the tomato cultivar Kecskeméti 555. 1. On the day of freezing; 2. on the 42nd day; 3. on the 77th day; 4. on the 117th day; 5. on the 161st day; 6. on the 202nd day; 7. on the 236th day; 8. on the 280th day; 9. on the 306th day; 10. on the 371st day of storage

Table 1

Correlation coefficients of the linear regression analyses between the colour characters of tomatoes at different degrees of ripeness and the storage period
(n = 8)

Colour character (CIELAB system)	Correlation coefficient (r)		
	1st degree	2nd degree of ripeness	3rd degree
a*	−0.893**	−0.978***	−0.991***
b*	0.933***	0.903**	0.999***
L*	0.951***	0.947***	0.990***
C_{ab}*	0.788*	−0.924**	−0.910**
h_{ab}°	0.901**	0.968***	0.998***
TCI	−0.975***	−0.970***	−0.995***

* Significant at P ≥ 95% probability level
** Highly significant at P ≥ 99% probability level
*** Very highly significant at P ≥ 99.9% probability level

Table 2

Correlation coefficients of the linear regression analyses between the colour characters of three different fully ripe tomato cultivars and the storage period
(n = 8)

Colour character (CIELAB system)	Correlation coefficient (r)		
	K1	K2	K3
a*	−0.614	−0.963***	−0.549
b*	0.819**	0.964***	0.902***
L*	0.907***	0.945***	0.900***
C_{ab}*	0.220	−0.249	0.402
h_{ab}	0.775*	0.985***	0.838**
TCI	−0.956***	−0.954***	−0.917***

* Significant at P ≥ 95% probability level
** Highly significant at P ≥ 99% probability level
*** Very highly significant at P ≥ 99.9% probability level

Table 3

Relationship between some colour characters (y) and the frozen storage period (x)

Sample		b*	L*	TCI
1st series	1st degree	y = 15.458 + 0.013x	y = 27.574 + 0.019x	y = 5.011 − 0.045x
	2nd degree	y = 14.572 + 0.016x	y = 25.490 + 0.013x	y = 8.040 − 0.018x
	3rd degree	y = 13.991 + 0.020x	y = 22.528 + 0.030x	y = 9.695 − 0.015x
2nd series	K1	y = 16.370 + 0.010x	y = 24.721 + 0.006x	y = 8.763 − 0.004x
	K2	y = 14.547 + 0.018x	y = 25.987 + 0.015x	y = 8.584 − 0.140x
	K3	y = 15.499 + 0.012x	y = 23.409 + 0.008x	y = 8.928 − 0.005x

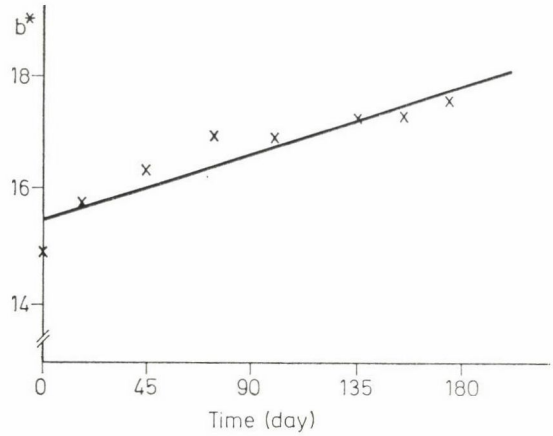


Fig. 7. Correlation between b* and storage period for a tomato sample at the 1st degree of ripeness (n = 8). r = 0.9327; y = 15.458 + 0.013x

In most cases a significant correlation was found between colour characters and storage time. The correlation between L* and TCI for all the samples was very close, for b* in four of the samples was very close, in 2 samples close. The equations are to be found in Table 3. To illustrate the change with time of the yellow character for b* the least ripe sample is illustrated in Fig. 7.

Table 4

Change of the carotenoid content in tomatoes of 1st degree ripeness during frozen storage related to the dry matter content
(μg per g)

Pigment		Storage period (day)							
		0	17	44	73	99	134	155	176
α-Carotene	$\bar{x}$	63.5	51.6	48.8	42.3	40.2	39.4	38.0	35.9
	s	2.0	1.2	2.1	2.0	2.2	2.1	1.0	3.1
β-Carotene	$\bar{x}$	84.6	81.7	78.4	60.7	52.0	47.1	38.7	34.9
	s	2.2	4.8	1.4	2.9	1.0	1.2	1.9	4.7
ζ-Carotene	$\bar{x}$	74.8	73.5	69.8	49.1	39.4	34.8	32.9	27.3
	s	2.0	4.1	1.3	1.1	2.1	1.0	2.2	2.2
φ-Carotene	$\bar{x}$	97.8	77.6	73.1	60.6	54.5	53.9	53.4	51.5
	s	3.0	2.0	4.7	2.3	2.3	3.8	4.1	1.4
Prolycopene	$\bar{x}$	86.9	70.2	65.3	52.5	48.9	42.9	41.4	40.5
	s	2.9	1.3	4.2	3.2	2.9	1.2	1.9	0.2
δ-Carotene	$\bar{x}$	53.9	52.2	51.5	49.1	40.9	37.4	30.5	28.5
	s	2.2	3.2	1.1	1.9	1.0	2.0	2.3	2.1
γ-Carotene	$\bar{x}$	358.5	254.4	196.5	192.9	165.5	156.9	135.9	135.9
	s	33.1	20.3	4.2	14.2	14.2	0.8	5.8	4.2
Neurosporene	$\bar{x}$	268.0	198.8	189.0	172.3	164.6	152.5	148.7	123.6
	s	18.3	15.3	0.9	2.4	2.1	4.2	10.8	10.8
Lycopene	$\bar{x}$	335.9	228.5	182.8	176.8	176.5	152.5	150.8	143.2
	s	8.0	17.8	13.1	4.1	8.3	2.3	2.4	10.4
Total		1423.9	1088.5	955.2	856.3	782.5	717.4	670.3	621.3

Table 5

Change of the carotenoid content in tomatoes of 2nd degree ripeness during frozen storage related to the dry matter content
(μg per g)

Pigment		Storage period (day)							
		0	20	42	70	97	132	153	174
α-Carotene	$\bar{x}$	64.8	54.6	52.7	52.7	44.9	42.1	36.0	32.7
	s	11.8	1.4	2.3	3.9	4.0	3.1	2.0	2.3
β-Carotene	$\bar{x}$	96.9	83.2	73.0	59.1	52.3	48.0	43.0	34.4
	s	2.2	4.1	4.2	12.7	2.1	2.2	3.1	0.8
ζ-Carotene	$\bar{x}$	80.6	71.8	65.4	61.7	54.8	47.5	44.1	26.3
	s	2.0	2.3	1.4	3.3	3.2	1.0	3.9	3.1
φ-Carotene	$\bar{x}$	110.7	90.0	87.2	74.4	57.7	52.4	50.6	46.2
	s	19.3	5.7	5.1	6.1	1.2	1.3	1.0	2.1
Prolycopene	$\bar{x}$	93.3	74.7	73.0	61.7	45.0	44.0	40.7	35.9
	s	17.1	4.9	2.1	5.9	1.4	2.0	1.3	1.4
δ-Carotene	$\bar{x}$	56.4	51.1	48.5	44.8	43.1	39.6	34.9	25.5
	s	2.3	1.3	1.0	3.2	3.0	3.1	3.2	2.0
γ-Carotene	$\bar{x}$	469.6	432.4	412.8	335.2	293.6	282.8	260.1	196.4
	s	37.2	44.7	55.7	2.0	10.9	25.1	6.3	7.2
Neurosporene	$\bar{x}$	421.7	362.1	335.0	329.2	311.8	265.7	261.9	213.3
	s	10.8	12.1	38.2	4.0	45.7	7.8	4.1	16.8
Lycopene	$\bar{x}$	480.0	368.5	360.1	341.4	307.9	287.6	252.7	246.8
	s	47.9	59.8	78.7	14.1	4.3	21.3	6.8	4.0
Total		1874.0	1588.4	1507.7	1360.2	1211.1	1109.7	1024.0	857.5

Table 6

Change of the carotenoid content in tomatoes of 3rd degree ripeness during frozen storage related to the dry matter content
(μg per g)

Pigment		Storage period (day)							
		0	8	43	76	98	133	154	175
α-Carotene	$\bar{x}$	68.1	58.5	53.2	49.3	48.0	46.6	37.8	29.2
	s	4.9	3.0	2.1	2.0	1.1	2.0	0.8	4.7
β-Carotene	$\bar{x}$	87.0	86.7	75.8	66.9	45.7	45.2	37.1	26.1
	s	5.1	2.4	0.5	4.1	2.0	2.1	0.9	3.0
ζ-Carotene	$\bar{x}$	72.1	65.9	62.7	62.8	61.4	53.8	50.7	44.9
	s	5.2	5.1	5.2	1.3	1.2	2.3	1.0	8.8
φ-Carotene	$\bar{x}$	117.3	96.7	92.4	70.1	59.5	45.5	44.6	38.6
	s	1.4	6.2	1.3	4.7	12.1	1.2	2.1	1.0
Prolycopene	$\bar{x}$	94.7	70.2	68.0	55.1	42.5	35.3	34.2	29.8
	s	6.8	2.9	2.4	0.6	5.8	1.0	1.3	1.2
δ-Carotene	$\bar{x}$	52.2	43.6	42.6	38.0	37.2	36.9	31.2	22.6
	s	4.0	0.4	2.0	1.2	1.4	1.1	0.7	3.0
γ-Carotene	$\bar{x}$	872.2	801.4	765.5	695.6	635.8	474.8	472.3	425.7
	s	27.8	41.7	42.3	0.9	65.6	9.2	13.0	21.8
Neurosporene	$\bar{x}$	622.4	614.2	596.0	592.6	583.3	572.4	489.0	484.9
	s	21.0	21.3	33.8	14.3	15.1	33.9	48.9	28.7
Lycopene	$\bar{x}$	737.1	697.0	672.4	623.3	587.2	574.7	558.8	545.5
	s	28.2	78.9	47.1	15.0	13.7	11.2	69.1	26.3
Total		2725.1	2534.2	2428.6	2253.7	2100.6	1885.2	1755.7	1647.3

Table 7

Correlation coefficients of the linear regression analyses between the carotenoid content of tomatoes at different degrees of ripeness and the storage period

Carotenoid	Correlation coefficient (r)		
	1st degree	2nd degree of ripeness	3rd degree
α-Carotene	−0.910**	−0.974***	−0.949***
β-Carotene	−0.987***	−0.974***	−0.984***
ζ-Carotene	−0.971***	−0.975***	−0.956***
φ-Carotene	−0.898**	−0.965***	−0.974***
Prolycopene	−0.943***	−0.961***	−0.950***
δ-Carotene	−0.975***	−0.967***	−0.920**
γ-Carotene	−0.877**	−0.982***	−0.986***
Neurosporene	−0.903**	−0.970***	−0.901**
Lycopene	−0.821*	−0.936***	−0.977***
Total	−0.922**	−0.983***	−0.994***

* Significant at $P \geq 95\%$ probability level
** Highly significant at $P \geq 99\%$ probability level
*** Very highly significant at $P \geq 99.9\%$probability level

It is striking that the equations describing the correlations of identical parameters with time, are similar. However the totalized analysis of results gave no good correlations. This shows that the change with time of the colour of tomatoes is dependent on variety and stage of maturity as well.

The steepness of the lines shows the change of colour with time to be a slow process.

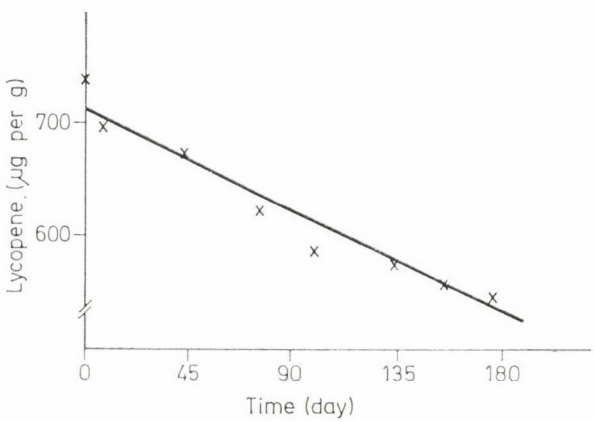


Fig. 8. Correlation between the lycopene content and storage period in a tomato sample at the 3rd degree of ripeness (n = 8). r = 0.9769; y = 714.2—0.010x

2.2. The quantity of carotenoids as a function of storage time

The carotenoid contents of the tomato samples at three different stages of maturity, kept for about 6 months in frozen storage, are shown in Tables 4, 5 and 6.

As it can be seen in the tables the quantity of carotenoids decreased with storage time. The retention of the pigments is highest in the tomatoes fully ripe. A close linear correlation was found between the carotenoid content and storage time in all the three samples frozen at different stages of maturity. (Table 7, Fig. 8).

The amounts of carotenoids in the samples of the ripe fruits of the three different cultivars, used in the second series are summarized in Tables 8, 9 and 10.

Here, too, the decrease of the carotenoid content with time is well visible. The correlation coefficients expressing the relationship are given in Table 11. In all the three cultivars a significant close correlation was found with gamma-carotene, neurosporene, lycopene and the total carotenoid content. These were the pigments present in the largest amounts in the samples. The correlation between the total carotenoid content and time is illustrated in Fig. 9.

2.3. Correlation between colour and carotenoid content

In the following the correlation between colour stimulus characters and the quantity of individual carotenoids was studied by linear regression analysis. The correlation coefficients found are shown in Tables 12 and 13.

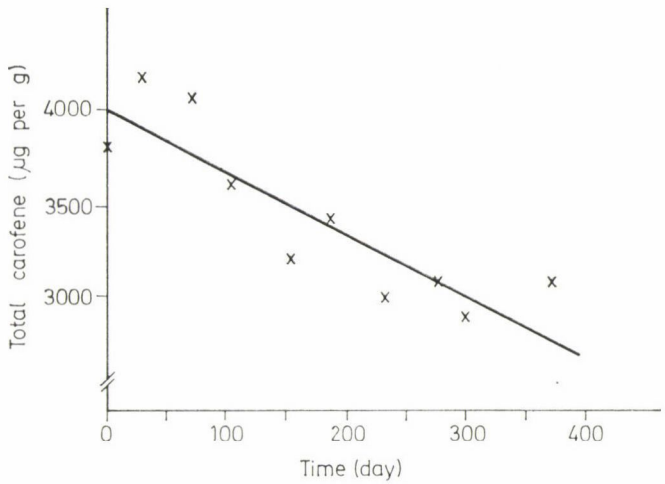


Fig. 9. Correlation between the total carotenoid content and storage period in tomato cultivar Kecskeméti 407 (n = 10). r = —0.869; y = 4003.90—3.32x

Table 8

Change in the carotenoid content of tomato cultivar Kecskeméti 407 (K1) during frozen storage, related to the dry matter content

Pigment		Storage period (day)									
		0	30	72	115	154	190	233	273	296	371
α-Carotene	$\bar{x}$	80.5	116.8	94.4	97.2	53.3	62.4	55.7	69.0	52.8	81.4
	s	13.2	6.3	12.1	25.2	2.1	28.1	2.8	11.5	5.0	17.0
β-Carotene	$\bar{x}$	77.6	111.3	93.0	91.6	55.4	66.0	60.5	68.6	22.4	81.0
	s	17.3	7.8	13.5	20.4	5.8	30.8	2.6	11.8	4.6	24.2
ζ-Carotene	$\bar{x}$	63.0	100.4	75.1	86.6	49.6	53.4	42.2	50.7	47.7	73.4
	s	8.8	6.5	12.9	24.9	11.1	23.6	2.3	10.8	2.6	13.8
φ-Carotene	$\bar{x}$	131.2	200.8	152.0	164.4	96.8	101.4	93.6	108.1	88.7	138.2
	s	20.6	11.1	11.6	42.7	9.2	45.4	2.8	21.7	6.0	30.0
Prolycopene	$\bar{x}$	10.9	163.8	130.2	135.4	73.4	87.7	73.9	95.3	70.3	112.0
	s	20.2	7.4	17.2	37.8	1.4	40.2	3.9	18.7	7.3	29.6
δ-Carotene	$\bar{x}$	66.7	93.5	75.7	76.4	44.0	51.1	46.1	56.7	39.5	65.9
	s	9.8	6.4	10.3	19.1	1.4	24.9	2.8	11.7	5.3	16.6
γ-Carotene	$\bar{x}$	1224.4	1331.0	1293.1	1117.3	1035.2	1149.3	972.3	1011.2	1038.9	959.1
	s	79.2	20.2	14.5	8.0	112.1	108.4	277.8	58.8	142.1	49.7
Neurosporene	$\bar{x}$	910.4	986.8	957.9	826.0	775.1	844.5	733.2	742.5	758.6	708.9
	s	63.2	16.7	13.8	3.5	78.9	66.2	210.7	47.7	106.2	31.2
Lycopene	$\bar{x}$	1158.4	1261.2	1201.3	1002.7	993.5	993.8	908.1	866.4	771.9	866.9
	s	118.4	27.5	16.6	8.4	115.9	87.6	282.2	40.0	131.0	103.4
Total		3823.1	4167.6	4072.7	3597.6	3176.3	3409.6	2985.6	3068.5	2890.8	3086.8

Table 9

Change in the carotenoid content of tomato cultivar Kecskeméti Jubileum (K2) during frozen storage, related to the dry matter content

Pigment		Storage time (day)									
		0	32	74	110	152	196	228	273	299	370
α-Carotene	$\bar{x}$	166.3	134.0	109.8	102.5	96.2	81.8	86.6	93.6	79.4	132.1
	s	0.6	12.5	6.7	4.6	20.1	4.7	11.8	17.8	14.1	7.6
β-Carotene	$\bar{x}$	164.4	138.9	114.6	104.6	99.3	83.2	88.0	93.3	32.0	100.4
	s	6.8	11.4	3.9	0.8	19.9	5.7	14.1	17.2	8.5	21.0
ζ-Carotene	$\bar{x}$	109.8	73.3	56.0	53.9	54.0	43.3	49.5	52.5	48.1	94.1
	s	3.6	14.4	5.3	9.0	22.5	14.2	8.3	9.3	10.4	11.7
φ-Carotene	$\bar{x}$	245.1	198.5	153.6	143.9	137.8	112.8	122.0	134.4	112.1	195.3
	s	3.2	16.1	8.3	7.5	33.1	9.3	18.1	27.7	22.1	19.7
Prolycopene	$\bar{x}$	214.5	183.6	145.9	140.4	125.4	109.3	116.0	125.6	105.3	181.8
	s	14.3	13.8	6.8	7.5	26.8	6.0	17.4	24.0	19.4	11.2
δ-Carotene	$\bar{x}$	135.4	112.0	88.9	79.8	78.4	65.9	68.8	75.1	61.0	104.6
	s	0.4	9.0	3.7	1.3	16.2	3.1	10.0	13.6	11.1	9.2
γ-Carotene	$\bar{x}$	939.5	784.9	725.0	566.0	468.2	410.6	372.4	304.2	307.0	342.0
	s	21.1	92.7	106.2	57.4	135.2	40.9	36.4	97.5	37.1	63.0
Neurosporene	$\bar{x}$	702.1	569.5	539.9	450.5	353.0	299.1	280.1	227.6	232.3	274.9
	s	19.4	81.8	81.8	37.8	105.3	33.3	30.3	75.5	29.6	51.5
Lycopene	$\bar{x}$	887.3	734.9	656.4	537.3	437.4	335.2	314.2	261.5	109.1	108.2
	s	25.5	97.9	98.4	39.2	129.7	36.4	36.8	87.5	32.2	2.7
Total		3564.4	2929.6	2590.1	2178.9	1849.7	1541.2	1497.6	1367.8	1086.3	1533.4

Table 10

Change in the carotenoid content of tomato cultivar Kecskeméti 555 (K3) during froze nstorage, related to the dry matter content

Pigment		Storage period (day)									
		0	42	77	118	161	203	236	280	307	371
α-Carotene	$\bar{x}$	77.1	86.7	82.6	69.1	85.9	55.0	59.2	63.1	59.8	80.0
	s	4.3	2.4	8.8	10.9	28.5	4.2	1.9	14.9	23.6	27.3
β-Carotene	$\bar{x}$	71.0	81.6	78.3	68.5	86.4	54.4	58.8	—	54.2	78.7
	s	2.1	0.7	7.4	7.0	34.7	4.6	1.3		20.0	27.6
ζ-Carotene	$\bar{x}$	71.0	95.8	107.7	72.4	100.2	50.6	62.3	61.4	67.0	76.8
	s	0.0	3.9	34.3	14.6	8.0	3.9	5.1	11.2	26.2	14.5
φ-Carotene	$\bar{x}$	137.5	164.3	155.5	135.3	158.6	98.6	107.7	114.0	110.7	143.7
	s	2.2	3.8	14.5	18.7	40.3	8.2	2.7	25.4	46.7	42.4
Prolycopene	$\bar{x}$	108.0	125.2	104.2	98.0	123.3	78.1	80.1	91.0	85.1	110.2
	s	5.4	2.6	9.3	15.8	39.4	3.9	3.1	19.9	36.2	39.7
δ-Carotene	$\bar{x}$	61.9	69.1	65.5	55.7	73.1	44.2	46.5	50.5	46.9	64.5
	s	2.1	0.8	7.0	8.4	24.5	3.7	1.0	12.7	18.6	23.3
γ-Carotene	$\bar{x}$	1054.3	1180.5	1140.0	912.8	948.3	818.4	829.5	782.6	865.8	792.3
	s	47.1	42.5	86.1	69.5	60.9	29.7	26.6	14.0	123.9	44.6
Neurosporene	$\bar{x}$	790.8	888.9	848.3	692.1	717.3	605.5	620.9	565.4	638.9	589.5
	s	33.1	31.1	57.4	54.4	54.4	24.4	18.3	13.9	83.7	32.5
Lycopene	$\bar{x}$	1020.4	1112.5	1064.7	882.3	848.1	725.2	733.2	385.3	716.3	588.7
	s	52.3	36.9	80.9	77.7	67.2	19.0	22.2	18.4	84.8	21.3
Total		3392.0	3804.6	3646.8	2986.2	3141.2	2530.0	2598.2	2113.3	2644.7	2524.4

Table 11

Correlation coefficients of the linear regression analyses between the carotenoid content in tomatoes of different cultivars and the storage period (n = 10)

Carotenoid	Correlation coefficient (r)		
	K1	K2	K3
α-Carotene	−0.560	−0.505	−0.466
β-Carotene	−0.561	−0.749*	−0.411
ζ-Carotene	−0.487	−0.252	−0.446
φ-Carotene	−0.555	−0.478	−0.504
Prolycopene	−0.566	−0.472	−0.441
δ-Carotene	−0.580	−0.541	−0.441
γ-Carotene	−0.861**	−0.926***	−0.851**
Neurosporene	−0.884***	−0.917***	−0.861**
Lycopene	−0.908***	−0.979***	−0.868**
Total	−0.869**	−0.864**	−0.846**

* Significant at P ≥ 95% probability level
** Highly significant at P ≥ 99% probability level
*** Very highly significant at P ≥ 99.9% probability level

In the first series of experiments with tomatoes at different stages of ripeness the correlation in almost all the cases was close and significant.

In the second series with three different cultivars significant correlation was found only between the carotenoids present in greatest amount (gamma-carotene, neurosporene, lycopene) and the total carotene content, on the one

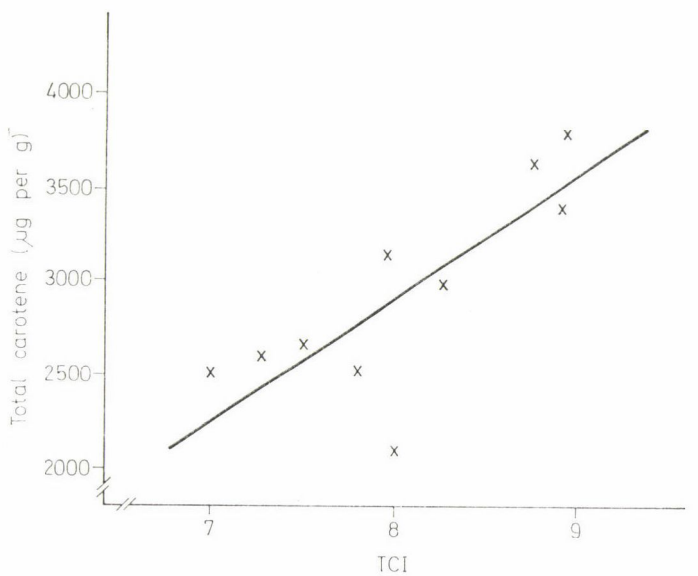


Fig. 10. Correlation between the total carotenoid content and the Tomato Colour Index in tomato cultivar Kecskeméti 555 (n = 10). r = 0.791; y = —2361.61 + 659.66x

Table 12

Correlation coefficients of the linear regression analyses between the colour characters of tomatoes frozen at different degrees of ripeness and their carotenoid contents (n = 10)

Pigment	Colour characters					
	a^*	b^*	L^*	C^*_{ab}	h°_{ab}	TCI
	1st degree of ripeness					
α-Carotene	0.929***	−0.906***	−0.841**	−0.803*	−0.947***	0.855**
β-Carotene	0.884**	−0.922**	−0.924**	−0.782*	−0.892**	0.960***
ζ-Carotene	0.894**	−0.924**	−0.885**	−0.760*	−0.900**	0.935***
φ-Carotene	0.928***	−0.978***	−0.806*	−0.778*	−0.945***	0.830*
Prolycopene	0.935***	−0.986***	−0.852**	−0.801*	−0.951***	0.880**
δ-Carotene	0.791*	−0.839**	−0.945***	−0.767*	−0.799*	0.960***
γ-Carotene	0.932***	−0.967***	−0.809*	−0.722*	−0.948***	0.804*
Neurosporene	0.885**	−0.969***	−0.871**	−0.850**	−0.908**	0.870**
Lycopene	0.897**	−0.949***	−0.757*	−0.729*	−0.917**	0.751*
Total	0.935***	−0.986***	−0.857**	−0.789*	−0.952***	0.864**
	2nd degree of ripeness					
α-Carotene	0.954***	−0.886**	−0.927***	0.901**	−0.944***	0.959***
β-Carotene	0.997***	−0.955***	−0.906**	0.921**	−0.993***	0.980***
ζ-Carotene	0.946***	−0.884**	−0.980***	0.868**	−0.939***	0.989***
φ-Carotene	0.984***	−0.915**	−0.879**	0.957***	−0.972***	0.948***
Prolycopene	0.980***	−0.909**	−0.885**	0.957***	−0.967***	0.949***
δ-Carotene	0.934***	−0.870**	−0.978***	0.859**	−0.927***	0.980***
γ-Carotene	0.979***	−0.903**	−0.953***	0.937***	−0.967***	0.980***
Neurosporene	0.961***	−0.919**	−0.930***	0.872**	−0.959***	0.981***
Lycopene	0.957***	−0.914**	−0.845**	0.902**	−0.951***	0.935***
Total	0.986***	−0.927***	−0.930***	0.928***	−0.978***	0.983***
	3rd degree of ripeness					
α-Carotene	0.967***	−0.924**	−0.920**	0.923**	−0.957***	0.964***
β-Carotene	0.970***	−0.979***	−0.977***	0.879**	−0.982***	0.987***
ζ-Carotene	0.950***	−0.934***	−0.923**	0.868**	−0.953***	0.959***
φ-Carotene	0.976***	−0.971***	−0.977***	0.917**	−0.977***	0.960***
Prolycopene	0.960***	−0.940***	−0.955***	0.924**	−0.953***	0.940***
δ-Carotene	0.941***	−0.903**	−0.980**	0.895**	−0.933***	0.943***
γ-Carotene	0.966***	−0.979***	−0.973***	0.870**	−0.979***	0.971***
Neurosporene	0.886**	−0.857**	−0.836**	0.806*	−0.891**	0.924**
Lycopene	0.984***	−0.969***	−0.982***	0.937***	−0.980***	0.970***
Total	0.986***	−0.981***	−0.952***	0.907**	−0.991***	0.989***

* Significant at $P \geq 95\%$ probability level
** Highly significant at $P \geq 99\%$ probability level
*** Very highly significant at $P \geq 99.9\%$ probability level

Table 13

Correlation coefficients of the linear regression analyses between the colour characters of frozen fully ripe tomatoes of different cultivars and their carotenoid contents (n = 10)

Pigment	Colour character					
	a*	b*	L*	C^*_{ab}	h°_{ab}	TCI
			Kecskeméti 407 (K1)			
α-Carotene	0.071	−0.229	−0.693*	−0.196	−0.176	0.485
β-Carotene	−0.082	−0.195	−0.699*	−0.379	−0.083	0.423
ζ-Carotene	0.003		−0.628	−0.128	−0.071	0.394
φ-Carotene	0.032	−0.182	−0.684*	−0.194	−0.130	0.458
Prolycopene	0.072	−0.232	−0.703*	−0.201	−0.178	0.492
δ-Carotene	0.060	−0.243	−0.711*	−0.230	−0 180	0.495
γ-Carotene	0.548	−0.605	−0.924***	−0.023	−0.614	0.872***
Neurosporene	0.536	−0.613	−0.937***	−0.050	−0.613	0.878***
Lycopene	0.399	−0.555	−0.930***	−0.166	0.406	0.813**
Total	0.428	−0.551	−0.922***	−0.113	−0.529	0.816**
			Kecskeméti Jubileum (K2)			
α-Carotene	0.352	−0.402	−0.430	0.183	−0.408	0.242
β-Carotene	0.630	−0.638*	−0.657*	0.351	−0.655*	0.540
ζ-Carotene	0.097	−0.217	−0.155	0.125	−0.151	−0.028
φ-Carotene	0.314	−0.440	−0.408	0.141	−0.376	0.213
Prolycopene	0.314	−0.426	−0.416	0.181	−0.371	0.205
δ-Carotene	0.378	−0.499	−0.473	0.154	−0.439	0.283
γ-Carotene	0.850**	−0.898***	−0.873***	0.265	−0.888***	0.795**
Neurosporene	0.848**	−0.879***	−0.858**	0.313	−0.880***	0.777**
Lycopene	0.921***	−0.930***	−0.907***	0.292	−0.946***	0.884***
Total	0.773**	−0.817**	−0.787**	0.308	−0.809**	0.741*
			Kecskeméti 555 (K3)			
α-Carotene	0.227	−0 288	−0.654*	−0.064	−0.323	0.512
β-Carotene	−0.240	−0.136	−0.460	−0.364	0.032	0.199
ζ-Carotene	0.103	−0.317	−0.670*	−0.236	−0.266	0.493
φ-Carotene	0.224	−0.347	−0.689*	−0.135	−0.357	0.551
Prolycopene	0.242	−0.319	−0.593	−0.085	−0.350	0.496
δ-Carotene	0.227	−0.261	−0.596	−0.040	−0.304	0.473
γ-Carotene	0.330	−0.782**	−0.982***	−0.491	−0.661*	0.863**
Neurosporene	0.311	−0.776**	−0.977***	−0.502	−0.647*	0.852**
Lycopene	0.182	−0.718*	−0.886***	−0.567	−0.537	0.737*
Total	0.245	−0.714*	−0.941***	−0.501	−0.574	0.791**

* Significant at $P \geq 95\%$ probability level
** Highly significant at $P \geq 99\%$ probability level
*** Very highly significant at $P \geq 99.9\%$ probability level

hand and the lightness factor (L*) and the Tomato Color Index, on the other. The correlation between the latter and the total carotenoid content is illustrated in Fig. 10. In two cultivars significant correlation was found between the same carotenoids and the b* value characteristic of yellow.

3. Conclusions

Of the change during frozen storage of the colour and carotenoid content in the same tomato cultivar at three different stages of ripeness (1st experimental series) and in the fully ripe fruits of three different cultivars (2nd series) the following were established:

The colour of tomato suffers continuous change during frozen storage. The extent of change depends on maturity. This becomes apparent in the diminishing of red character and increase of the yellow character, while the sample becomes lighter. Saturation does not undergo a characteristic change.

The change in colour shows linear correlation with storage time. For the values b*, L* and TCI this correlation is close and significant.

The quantity of carotenoids decreases during frozen storage. The diminishing of the pigments present in the samples and the total carotenoid content is in close linear correlation with storage time.

The quantity of the same pigments shows a close correlation with colour stimulus characters b* and L* and the Tomato Color Index. In consequence on the basis of the relatively more easily determinable colour characters conclusions can be drawn not only as to colour changes, but to the quantitative changes of these pigments, too.

Literature

Bontovits, L. (1960): A paradicsom színe. (Colour of tomatoes.) *Élelmiszerv. Közl.*, *6*, 12–21.

Bontovits, L. (1974): A színmérés kérdései a paradicsom minősítésénél. (Problems of colour measurement in tomato qualification.) *Konzerv Paprikaipar*, Special issue, 36–40.

Bontovits, L. (1979): Az előkészítési eljárás hatása a paradicsomlé minták színalakulására. (The colour of tomato juice as affected by the technique of pretreatment.) *Konzerv Paprikaipar*, 125–128.

Bontovits, L. (1980): A paradicsom színmérése, mint a gyártásirányítás és készletgazdálkodás lehetősége. (Measurement of tomato colour as a possibility to control processing and stockpiling.) *Konzerv Paprikaipar*, 146–148.

Dixon, T. S. & Hobson, G. E. (1984): A general method for the instrumental assessment of the colour of tomato fruit during ripening. *J. Sci. Fd Agric.*, *35*, 1277–1281.

Drdak, M. Bielicka, D. & Pribela, A. (1977): Meranie farby rajcionovych pretlakov. (Measuring the colour of tomato puree.) *Prum. Potravin.*, *28*, 529–531.

Drdak, M. & Sorman, L. (1979): Farbe von Tomatenpurees und ihre Veränderungen während der Lagerung. *Ind. Obst Gemüseverwert.*, *64*, 327–330.

Francis, F. J. & Clydesdale, F. M. (1975): *Food colorimetry: theory and applications.* The AVI Publishing Co., Westport, p. 194.

JEN, J. J. (1974): Carotenoids of yellow and red lutescent tomatoes. *J. agric. Fd Chem.*, *22*, 908–910.

HALL, C. B. (1961): The effect of low storage temperatures on the colour, carotenoid pigments, shelf-life and firmness of ripened tomatoes. *Proc. Am. Soc. hort. Sci.*, *78*, 480–487.

HARKAY, T. & BONTOVITS, L. (1981): A TOMACOLOR paradicsom színminősítő műszer alkalmazási tapasztalatai. (Experiences gained with the TOMACOLOR tomato colour measuring instrument.) *Konzerv Paprikaipar*, 10–13.

KOSKITALO, L. N. & ORMROD, D. P. (1972): Effects of sub-optimal ripening temperatures on the colour quality and pigment composition of tomato fruit. *J. Fd Sci.*, *37*, 56–59.

NATTUVETTY, V. R. & CHEN, P. (1980): Maturity sorting of green tomatoes based on light transmittance through regions of the fruit. *Trans. ASAE*, *23*, 515–518.

NOBLE, A. C. (1975): Investigation of the colour changes in heat concentrated tomato pulp. *J. agric. Fd Chem.*, *23*, 48–49.

RAYMUNDO, L. C., CHICHESTER, C. O. & SIMPSON, K. L. (1976): Light-dependent carotenoid synthesis in the tomato fruit. *J. agric. Fd Chem.*, *24*, 59–64.

THOMPSON, A. E. (1961): A comparison of fruit quality constituents of normal and high pigment tomatoes. *Proc. Am. Soc. hort. Sci.*, *78*, 464–473.

THOMPSON, A. E., TOMES, M. L., WANN, E. V., MCCOLLUM, J. P. & STONER, A. K. (1965): Characterization of crimson tomato fruit colour. *Proc. Am. Soc. hort. Sci.*, *86*, 610–616.

THOMPSON, A. E., TOMES, M. L., ERICKSON, H. T., WANN, E. V. & ARMSTRONG, R. J. (1967): Inheritance of crimson fruit colour in tomatoes. *Proc. Am. Soc. hort. Sci.*, *91*, 495–504.

TOMES, M. L. (1963): Temperature inhibition of carotene synthesis in tomato. *Bot. Gaz.*, *124*, 180–185.

WATADA, E. E., NORRIS, K. H., WORTHINGTON, J. T. & MASSIE, D. R. (1976): Estimation of chlorophyll and carotenoid contents of whole tomato by Light Absorbance Technique. *J. Fd Sci.*, *41*, 329–332.

WORTHINGTON, J. T. (1971): A light-transmittance technique for determining tomato ripening rate and quality. *Acta Hortic.*, *38*, 193–215.

WORTHINGTON, J. T., MASSIE, D. R. & NORRIS, K. H. (1973): Light transmission technique for predicting ripening time for intact green tomatoes. *Am. Soc. Agric. Eng.*, Paper No. 73–6526, 1–5.

YEATMAN, J. N. (1969): Tomato products: Read tomato red? *Fd Technol.*, *23*, 618–627.

YEATMAN, J. N. (1976): Tomato colour standards need revision. *Fd. Technol.*, *30*, (11), 50–56.

Acta Alimentaria, Vol. 18 (3), pp. 269—282 (1989)

ADAPTATION OF COLLABORATIVE METHODOLOGY TO THE SENSORY EVALUATION OF FOODS BY SCORING

P. MOLNÁR

Institute of Food Control, H-1095 Budapest, Mester u. 81. Hungary

(Received: 23 March 1988; accepted: 21 November 1988)

Random error appearing in the course of food analysis can be determined by collaborative tests. In the collaborative test latent paired samples are investigated by the same method under similar circumstances and conditions by several laboratories. The results of the investigation tested by different statistical techniques are interpreted by two-way analysis of variance. This interpretation permits the determination according to ISO 5725 of the tolerable fluctuation of results obtained within laboratory: repeatability (r), and the permissible deviation of the analytical results between laboratories: reproducibility (R).

Collaborative sensory evaluation is a specific case of collaborative study. Laboratories are replaced by sensory panels. The weighting factor method of sensory evaluation by scoring each group of attributes on a 5 point scale with a total of 20 points proved suitable for collaborative study. Data obtained in the collaborative test permitted of drawing conclusions as to the suitability of the sensory panels and the level and unambiguity of interpretation of the specifications. By interpreting the experiences of the collaborative studies and by the summing up of the findings the evaluation method was revised and made more precise and the pertinent methodological standard was updated. The r and R values obtained in the sensory evaluation by scoring of soft drinks can be utilized in quality control.

Keywords; sensory collaborative study, scoring, sensory evaluation

At present none of the methods used in food analysis provide absolutely precise and perfectly reproducible results. Even in the case of the most careful work in instrumental analyses as well as in sensory evaluations carried out in several repetitions complete identity of results occurs only randomly.

Two main factors of error are considered in related literature: sampling error and systematic error of the method. The knowledge of the systematic error beside other characters of the method (rapidity, requirement of material and instruments, etc.) is an important condition of solid quality control. Thus, the differences between results obtained in inter-laboratory measurements of uniform samples and those obtained in repeated within-laboratory study of the same samples, which may occur due to uncertainties in the method, have to be known.

The character and extent of random error occurring in the course of testing the sample may be determined by collaborative test. In the course of the collaborative study latent paired samples are studied by several laboratories

under identical circumstances and by the same method. Data of the study are interpreted by two-way analysis of variance. This interpretation serves to determine, in accordances with INTERNATIONAL STANDARDS ORGANIZATION (1981), the within-laboratory tolerable fluctuation of results: repeatability (r), as well as the inter-laboratory permissible differences of results: reproducibility (R).

Collaborative sensory evaluation is a specific case of analytical collaborative study. This divergent character may be traced back partly to, although decreasing, however existing differences in taste among various countries, regions, geographical units, partly to methodological reasons. From the point-of-view of methodology two basically differing types of collaborative sensory evaluations exist:

— In the case of the descriptive collaborative test of sensory evaluations the food sample or other model material (e.g. pectin jelly) prepared specially for this purpose and of different quality or set characteristic (e.g. different consistency or flavouring) are sent to the collaborant laboratories. The task of the respective panels is to give an exact description of the sensory properties of the sample or model material, taking into account the local food habits, the general taste and the evaluation of the public opinion.

— The determination of random error in sensory evaluation methods — similarly to that of analytical methods — can be carried out only on the basis of numerically expressed results. Therefore, in the case of this type of sensory collaborative test decisive role is attributed to the construction of the applied scoring method, inferring the use of a symmetric, nearly proportional scale. This requirement is considerably met by the weighting factor method of scoring each group of attributes on a 5 point scale out of a total of 20 points (MOLNÁR, 1983). Thus, on interpreting the results the starting data are the average scores for each group of attributes obtained by the sensory panel. Accordingly, repeatability and reproducibility are obtained for each group of attribute and the repeatability and reproducibility pertinent to the weighted total scores can be calculated by the method of Gauss.

In this paper we wish to present the preparation, conditions, interpretation of data and the results of the sensory collaborative test based on the scoring method.

1. Methodology of the sensory collaborative test

To carry out sensory collaborative studies the same circumstances characterizing generally the sensory evaluations are required. Otherwise, the applicability of the results obtained is doubtful.

1.1. Sensory evaluation method

The sensory collaborative tests were carried out by methods as characterized in the introduction,

— mostly well known to and practised by panel members,

— by the aid of which the data obtained by sensory evaluation (average score for each group of attributes) were of normal distribution and the homogeneity of scatter was approximately guaranteed, while the data could be considered independent of one another.

1. 2. Samples for the collaborative test

The samples selected for collaborative test were in every case representative of the group of products to which the evaluation specifications pertained. Great care was taken to ensure the homogeneity of the samples sent out to the participating panels, however, this was not always successful. Likewise, it was difficult to ensure samples at different levels of quality. However, the problem was more or less resolved by storing them for a longer period at room temperature. By this method, however, only certain sensory defects could be "presented". If in the course of evaluation parallel samples were recognisable by one of their characteristics (e.g. colour or form) in that case slightly different, so called "troubling" samples were included in the evaluation program.

Within one collaborative program each participating panel obtained generally 5–8 different samples, every one of the samples twice. Treating the parallel samples individually each panel evaluated in every program 10–18 samples, perhaps in addition 1–3 "troubling" samples. When every mark of identification was removed from the samples for every panel they were coded with an individual three digit number. The closing and packaging of the samples carried out so that their quality should possibly be prevented from change during transport or storage.

Thus, every participating panel evaluated on the same day 11–21 samples in accordance with the general specifications for the given product (HUNGARIAN STANDARD, 1977; 1986a).

1.3. Panel members of the collaborative test

The leaders of the collaborative test selected the members of the panel in accordance with the character of the product. Each panel consisted of at least 5 members who had to meet the following requirements:

— basic suitability to sensory evaluation in accordance with the respective HUNGARIAN STANDARD (1982a; 1980; 1982b; 1982c);

— exact knowledge of the evaluation method;

— knowledge of the product;

— regular practice in evaluation;
— the highest possible objectivity.

In selecting the panel the environmental circumstances affecting objectivity of evaluation (appropriate room for preparation and judging) were also kept in view. In each collaborative program at least 4 panels participated.

1.4. Guide to the collaborative study

The guide provided for panel members contained the following:
— the time, mode and circumstances of presenting, transporting and storage of the samples;
— date of the sensory evaluation;
— number, coding and order of evaluating the samples to be tested;
— specifications of the sensory method applied;
— the observable circumstances of evaluation (e.g. way of preparing the sample: thawing, heating, roasting, boiling, etc. and the temperature of the sample judged);
— further information related to the performance and preliminary evaluation (e.g. completing of the evaluation form, mode of data supplying, calcuation of the average score, calculation of the weighted total score);
— table for the summarized results of the evaluation (average score for the attribute-groups, weighted total scores);
— latest date for sending of the results;
— a general invitation to send in any suggestions as to the solution of difficulties arising in the course of evaluation or to the revision or correction of the specifications of evaluation.

1. 5. Statistical evaluation and interpretation of the collaborative test

Interpretation of the results of the collaborative test was carried out under the direction of the current leader of the test. Namely, it seemed often desirable to give professional interpretation of results and define the task ensuing beyond the mathematical-statistical interpretation (e.g. stricter testing of outlier values).

The results of scoring the "troubling" samples were, naturally, not included in the interpretation. It is expedient to use a personal computer for the interpretation of data. The program made for a Commodore 64 accelerated interpretation and eliminated sources of error.

1.5.1 Symbols used in the interpretations

i : index of the panel $\quad i = 1 \ldots p$
L_i : symbol of the panel
j : index of the samples $\quad j = 1 \ldots q$

M_j : symbol of the samples
p : number of panels
q : number of samples
$2\,pq$: total number of data
$A, B, C, D, \ldots N$: symbol of the attribute-groups
T : symbol of the total score
Y_{ij1} : first score given to an attribute-group of sample j given by panel i (average score)
Y_{ij2} : second score given to an attribute-group of sample j by panel i (average score)
a_{ij} : sum of the scores (average scores) given to an attribute-group of sample j by panel i,: $Y_{ij1} + Y_{ij2}$
w_{ij} : difference of connected data pairs: $Y_{ij1} - Y_{ij2}$
G : sum of all the data evaluated in relation to an attribute-group
g_j : sum of the connected data pairs of a sample formed from the judgement of all panels
h_i : sum of the tested data pairs of a panel formed from the evaluation of every sample
d : average of the deviations from the common average score of the samples evaluated in parallel by the individual panels
M : correction factor to form the sum of squares
MS : medium square deviation
SS : deviation sum of squares
SS_{Aa}: deviation sum of squares of the summarized data pairs in attribute-group "A"
SS_{BA}: deviation sum of square of the summarized data pairs in attribute-group "B"
SS_{Ai} : deviation sum of squares of panels in attribute-group "A"
SS_{Aij}: deviation sum of squares of interaction between samples and panels in attribute-group "A"
SS_{Aj} : deviation sum of squares of samples in attribute-group "A"
SS_{Ar} : sum of squares of repeats and parallel evaluations in attribute-group "A"
e : number of estimated pairs
DF : degree of freedom
s_{Ai}^2 : variance among panels for attribute-group "A"
s_{Ti}^2 : variance among panels summed up for total score
s_{Ar}^2 : variance of results of evaluations obtained under conditions of repeatability for attribute-group "A"
s_{Br}^2 : variance of results of evaluations obtained under conditions of repeatability for attribute-group "B"
s_{Tr}^2 : variance of results of evaluations obtained under the conditions of repeatability summed up to the total sensory score

s_{Aij}^2 : variance of the interaction between panels and samples for attribute-group "A"

s_{Bij}^2 : variance of the interaction between panels and samples for attribute-group "B"

s_{Tij}^2 : variance of the interaction between panels and samples summed up for the total sensory score

SF_A : weighting factor of attribute-group "A" in forming the total sensory score

r_A : repeatability of attribute-group "A"

r_T : repeatability of the total score

R_A : reproducibility of attribute-group "A"

R_T : reproducibility of the total score

Q_{crit} : critical value of the Dixon test

Q_{cal} : calculated value of the Dixon test

s_A : scatter of the difference of two mean values for attribute-group "A"

1.5.2 Summing up of the results in a table. The results of the sensory evaluations according to attribute-groups is summed up in Table 1.

Table 1

Average scores of "Appearance" (A) determined in parallel by samples and panels

Panel	Sample				
	M_1	M_2		M_j	M_q
L_1	Y_{111} Y_{112}	Y_{121} Y_{122}	....	Y_{1j1} Y_{1j2}	Y_{1q1} Y_{1q2}
L_2	Y_{211} Y_{212}	Y_{222} Y_{222}	....	Y_{2j1} Y_{2j2}	Y_{2q1} Y_{2q2}
. . .	. . .	. . .	....	. . .	. . .
L_i	Y_{i11} Y_{i12}	Y_{i21} Y_{i22}	....	Y_{ij1} Y_{ij2}	Y_{iq1} Y_{iq2}
L_p	Y_{p11} Y_{p12}	Y_{p21} Y_{p22}	....	Y_{pj1} Y_{pj2}	Y_{pq1} Y_{pq2}

1.5.3. Tests of the preconditions of analysis of variance. Although the probability of the occurrence of outstanding values as regards normal distribution and homogeneity of scatter is low due to the mean-value character of results of sensory evaluations, it is expedient to test by various statistical techniques the pre-conditions of analysis of variance. In doing this in every case, the 95% probability was taken as a basis.

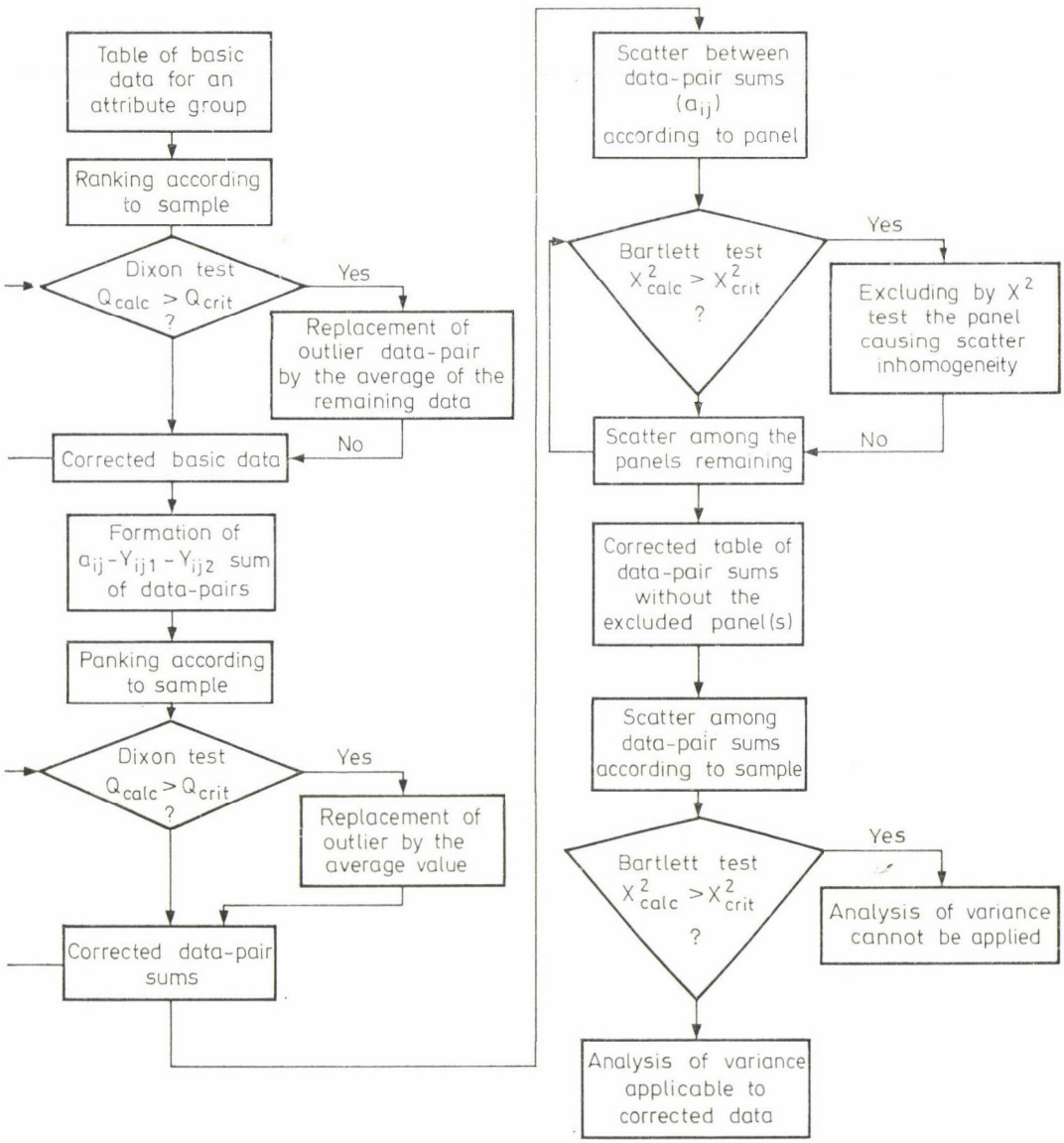


Fig. 1. Scheme of testing the pre-conditions of analysis of variance by sensory collaborative study

Bias influencing repeatability were sifted out for the average score, for each group of attributes by the Dixon test. To be able to do this the average score belonging to a given sample were ranked. The statistical test of outliers was carried out according to DIXON (1953) by different equations dependent on the number of data. The Dixon test was applied for each sample to the

data-pair sums (a_{ij}), too. Then the significant differences affecting calculation of repeatability were excluded from further interpretation. The excluded values were estimated by the mean values belonging to the remaining scores in order to have sufficient data and thus they would lend themselves to interpretation in the same way. In accordance to related literature (GOTTSCHALK & KAISER, 1976) this technique is acceptable since the scatter reducing effect is negligible if the number of estimated data does not exceed the proportion of 5%. Anyway, in the case of sensory collaborative study there is no other way of complementing data. In determining the number of degrees of freedom (DF) the estimated data (e) were taken into account.

The Bartlett test was used to check, according to SACHS (1978) the homogeneity of the sum of data-pairs and was carried out on each sample and each panel.

The block diagram of the statistical methods generally applied to test pre-conditions of analysis of variance, is shown in Fig. 1.

Data in relation to outlier values of repeats were investigated according to DÖRFEL (1966) by the Cochran test. This was carried out by forming the square sum of differences between average scores of parallels and presented in a table. The quotient of the highest difference of squares (w_{ijmax}) and the sum of all the squares of differences was compared depending on the number of data-pairs with related tabulated data. The outliers, here too, were replaced by the average value of remaining scores. If all, or almost all of the scores of a given panel are outliers their evaluation is erased.

In some of the cases the difference between average scores, appearing striking from the professional point-of-view, did not prove significantly deviating. Therefore, in the interpretation of the second collaborative study of soft drinks (SZABÓ et al., 1987), to check the homogeneity for each sample of the set of data, instead of the less sensitive Dixon test the r test was used (GOTTSCHALK & KAISER, 1976).

In the r test the following value is formed:

$$PG_i = \frac{(a_{ij} - \bar{a}_j)}{s} \cdot \sqrt{\frac{q}{q-1}}$$

where

a_{ij}: sum of average scores
$\bar{a}_j$: average of the average score of the sample
s : standard deviation of the data investigated (a_{ij})
q : number of samples.

The PG_i value is compared to the r value in the table and then the value investigated is

— non outlier,
— probably outlier,
— significantly outlier,
— highly significantly outlier.

According to the rules given for applying the test, in this case, too, the outlying data were replaced by the average value of the remaining scores. The test has to be repeated till another outlier is found. The use of the r test proved advantageous because among the outlier tests the probability of making a secondary mistake, that is retaining an outlier, is the lowest with this method. The listing of the "probably outlier" values was decided on the basis of professional considerations and these were treated mostly like outliers.

1.5.4. Analysis of variance. The table of the sums of the average scores was already prepared for carrying out the control tests (Table) where $a_{ij} = Y_{ij1} + Y_{ij2}$.

Table 2

Sum of the average scores of "Appearance" (A) for each sample and each panel

Panel	Sample					
	M_1	M_2		M_j	M_q	Sum
L	a_{11}	a_{12}	....	a_{ij}	a_{iq}	h_1
L_2	a_{21}	a_{22}	....	a_{2j}	a_q	h_2
.	.	.		.	.	.
.	.	.		.	.	.
.	.	.		.	.	.
L_i	a_{i1}	a_{i2}	....	a_{ij}	a_{iq}	h_i
L_p	a_{p1}	a_{p2}	....	a_{pj}	a_{pq}	h_p
Sum	q_1	q_2	....	q_j	q_q	G

To fill in the table of variances the following deviation square sums were calculated. The related calculation processes are given for the attribute-group "A" as an example:

For samples $$SS_{Aj} = \sum_{j=1}^{q} \frac{q_i^2}{2p} - M$$

For panels $$SS_{Ai} = \sum_{i=1}^{p} \frac{h_i}{2q} - M$$

For data-pairs $$SS_{Aa} = \frac{1}{2} \sum_{i=1}^{p} \sum_{j=1}^{q} a_{ij}^2 - M$$

For repeats $$SS_{Ar} = \frac{1}{2} \sum_{i=1}^{p} \sum_{j=1}^{q} w_{ij}^2$$

Correction factor $$M = \frac{G^2}{2pq}$$

Interaction between panels and samples $$SS_{Aij} = SS_{Aa} - SS_{Ai} - SS_{Aj}$$

Variances required to calculate repeatability and reproducibility were summed up as presented in Table 3 for each collaborative study.

Table 3

Table of variance belonging to attribute group "A"

Factor	*SS* values	*DF* values	*MS* values
Panel	SS_{Ai}	$p - 1$	$S^2_{Ai} = \frac{SS_{Ai}}{p-1}$
Repeat	SS_{Ar}	$pq - e$	$S^2_{Ar} = \frac{SS_{Ar}}{pq-e}$
Interaction	SS_{Aij}	$(p-1)(q-1)-e$	$S^2_{Aij} = \frac{SS_{Aij}}{(p-1)(q-1)-e}$

1.5.5. Calculation of repeatability and reproducibility. Repeatability for attribute group "A" was calculated from the square of deviations of the sensory scores obtained under the conditions of repeatability:

$$r_A = t_{0.05} \sqrt{2s^2_{Ar}}$$

Repeatability of the weighted total score can be calculated by the following two equations:

$$r_T = t_{0.05} \sqrt{2s^2_{Tr}}$$

where

$$s_{Tr} = \sqrt{s^2_{Ar} SF^2_A + s^2_{Br} SF^2_B + \ldots}$$

Reproducibility for attribute group "A" was calculated from squares of deviation s^2_{Ar}, s^2_{Ai}, s^2_{Aij} by the following equations:

$$R_A = t_{0.03}\, s_{Ad} \text{ where } s_{Ad} = \sqrt{s^2_{Ar} + \frac{i}{q} s^2_{Ai} + \left(1 - \frac{1}{q}\right) s^2_{Aij}}$$

$$R_A = t_{0.05} \sqrt{s^2_{Ar} + \frac{1}{q} s^2_{Ai} + \left(1 + \frac{i}{q}\right) s^2_{Aij}}$$

Reproducibility of the weighted total score can be calculated by the following equations:

$$R_T = t_{0.05} \sqrt{s^2_{Tr} + \frac{1}{q} s^2_{Ti} + \left(1 - \frac{1}{q}\right) s^2_{Tij}}$$

where

$$s_{Ti} = \sqrt{s^2_{Ai} SF^2_A + s^2_{Bi} SF^2_B + \ldots}$$

and

$$s_{Tij} = \sqrt{s^2_{Tij} SF^2_A - s^2_{Bij} SF^2_B + \ldots}$$

2. Results

The developed collaborative study was first applied in Hungary to soft drinks.

The collaborative sensory evaluation of soft drinks based on Hungarian fruits including grapes and on citrus fruits was carried out on occasions. In the second collaborative study the improved specifications were used (HUNGARIAN STANDARD, 1986). The results of this study were described in another paper, too (SZABÓ et al., 1987).

The first investigation was carried out as an intra-laboratory study, meaning thereby that the participating panels tasted the samples at the same institution in parallel tests.

The number of participating panels was 5 and the number of the samples 8.

The second sensory collaborative test was carried out as an interlaboratory investigation meaning thereby that the panels evaluated the samples under customary conditions in their own rooms.

The number of participating panels was 13 and the number of soft drink samples 4.

In the course of the first collaborative evaluation of the 40 data-pairs for each attribute group 2 data-pairs for "aroma" were dropped as outliers using the Cochran test, while neither the Dixon nor the Bartlett test gave significantly differing values. In the second collaborative evaluation out of the 52 data-pairs for each attribute group 6 were found outliers by the Cochran test, one data-pair each for "appearance" and "aroma" and two for "colour" and "taste", respectively. When using the x^2 test of Kaiser and Gottschalk with "appearance" two, with "colour" five, with "aroma" eight and with "taste" five data-pairs were found outliers. The increase to this extent of the number of outliers was traced back to the following reasons:

— introduction of stricter statistical tests;

— higher inhomogeneity of the samples because of the wider range of quality;
— application of a more differentiated evaluation scale.[1]

Evaluation of the first (I) and second (II) collaborative trials lead to the results for all panels and samples presented in Table 4.

Table 4

Repeatability (r) and reproducibility (R) of the sensory evaluation by scoring of soft drinks

Attribute group	SF	r		R	
		I	II	I	II
Appearance	0.6	0.4	0.5	0.6	0.8
Colour	0.6	0.6	0.5	0.7	0.9
Aroma	0.8	0.8	0.8	1.3	1.1
Taste	2.0	0.9	0.8	1.2	1.1
Weighted total score	—	2.0	1.8	2.7	2.5

It should be noted that the presumably higher inhomogeneity of the samples in the second collaborative trial is shown also by the higher r and R values calculated in relation to "appearance". It is shown also by the decrease of average standard deviations of the scores given by the panels in the second collaborative trial in comparison to the first one, except those of "appearance" and it proves that the specifications were updated in the right direction.

3. Conclusions

By the development of the sensory collaborative test the methodological fundament and evaluation technique of the food analytical collaborative study, were adapted to sensory evaluation. This technique, hitherto not published in related literature, opens new possibilities for the sensory evaluation methods by scoring to be tested in many different ways.

The sensory collaborative trial of soft drinks has unambiguously proven the applicability and reliability of the developed collaborative technique. The results obtained reflect rightly the possibilities of the applied scoring method and are acceptable also from the viewpoint of practice. Similar data were published in the dissertation of KOCHAN (1976).

The sensory collaborative test of the soft drinks permits the following conclusions:

— the evaluation of "aroma" and "taste" contains a substantially higher uncertainty factor than that of the visual attributes. The pertinent r and R values, however, very substantially reduced in the second collaborative study.

— In the second test the r and R values of visual attributes were higher. This was certainly not caused by reduced accuracy of scoring but by the greater inhomogeneity of the visual attributes of the samples, causing, as is well known, the increase of random error of the method.

— The values of repeatability — except for the "colour" — exceed by far the half of the values of reproducibility, which is considered with simple food analytical methods — and thus here, too — a generally accepted rule. This shows also that with the expectable improvement of the repeatability values of individual attribute groups the r value of the weighted total score can be further reduced.

Experiences gained with sensory collaborative tests of soft drinks confirmed the hypothesis that the symmetry in the construction and structure of the sensory scoring method is the condition of the comparability of the r and R values. If this condition is met the repeatability and reproducibility as determined by the comparative test, can be used in the comparison of the results of Hungarian, foreign and international quality control by sensory scoring. Sensory collaborative tests may play an important role in the testing of evaluation specifications and description of methods, in their conciliation and in increasing their accuracy, in the co-ordination of the work of panels, in the unifications of the scale of judgement and in carrying out the sensory tests correctly. This expectation was proven by the results of the repeated collaborative tests.

The methodology of the collaborative study is tested by the sensory evaluation of other products of the food industry. Results of this investigations will be published in another paper.

Literature

DIXON, W. I. (1953): Processing data for outliers. *Biometrics, 9*, 74–84.

DÖRFEL, K. (1966): *Statistik in der analytischen Chemie.* Deutscher Verlag der Grundstoffindustrie, Merseburg, pp. 68–78.

GOTTSCHALK, G. & KAISER, R. E. (1976): *Einführung in die Varianzanalyse und Ringversuche.* Bibliographisches Institut Mannheim, Wissenschaftverlag, pp. 74–81.

HUNGARIAN STANDARD (1977): *Érzékszervi vizsgálati módszerek. Előkészítő helyiség, bírálati helyiség.* (Sensory testing methods. Preparatory room, test room.) MSZ 7304/2.

HUNGARIAN STANDARD (1980): *Élelmiszerek érzékszervi vizsgálati módszerei. Ízküszöbérték vizsgálata.* (Methods of sensory analysis of foodstuffs. Test of the threshold value of flavour.) MSZ 7304/6.

HUNGARIAN STANDARD (1982a): *Élelmiszerek érzékszervi vizsgálati módszerei. Izlelőképesség vizsgálata.* (Methods of sensory analysis of foodstuffs. Test of tasting ability.) MSZ 7304/1.

HUNGARIAN STANDARD (1982b): *Élelmiszerek érzékszervi vizsgálati módszerei. A szaglóképesség vizsgálata.* (Methods of sensory analysis of foodstuffs. Test of olfactive ability.) MSZ 7304/10.

HUNGARIAN STANDARD (1982c): *Élelmiszerek érzékszervi vizsgálati módszerei. Színmegállapító képesség vizsgálata.* (Methods of sensory analysis of foodstuffs. Test of the ability to determine colours.) MSZ 7304/12.

HUNGARIAN STANDARD (1986a): *Élelmiszerek érzékszervi vizsgálati módszerei. Általános előírások.* (Methods of sensory analysis of foodstuffs. General specifications.) MSZ 7304/3.

HUNGARIAN STANDARD (1986b): *Alkoholmentes üdítőital. Ézékszervi pontozásos bírálat.* (Non alcoholic beverages. Sensory analysis.) MSZ 21338/2.

HUNGARIAN STANDARD ORGANIZATION (1981): *Precision of test methods — Determination of repeatability and reproducibility by inter-laboratory test.* ISO 5725.

KOCHAN, A. (1976): Problemlösungen zur Qualitätssicherung in der Lebensmittelindustrie und Nahrungsgüterwirtschaft. Dissertation, Technische Universität Dresden.

MOLNÁR, P. (1983): *Élelmiszerek érzékszervi minősítése I. Pontozásos bírálat.* (Sensory evaluation of foods. I. Evaluation by scoring.) MÉM Mérnök- és Vezetőtovábbképző Intézet, Budapest, pp. 19–50.

SACHS, L. (1978): *Angewandte Statistik — Statistische Methoden und ihre Anwendungen.* Springer Verlag, Berlin, pp. 384–416.

SZABÓ, É., MOLNÁR, P., GÓLYA, Zs. & ÁCS, P. (1987): Érzékszervi körvizsgálat eredményei üdítőitaloknál. (Results of sensory collaborative study with soft drinks.) *Élelmiszerv. Közl., 33,* 137–146.

Acta Alimentaria, Vol. 18 (3), pp. 283—297 (1989)

A COMPARATIVE STUDY OF THREE PROCEDURES FOR THE ISOLATION OF VOLATILE FLAVOUR COMPOUNDS FROM A MODEL SOLUTION

M. J. GAGNON and A. M. MARCOUX

Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Chicoutimi, Québec. Canada G7H 2B1

(Received: 3 May 1988; accepted: 10 November 1988)

Three methods to isolate a mixture of volatile flavour compounds from an aqueous model solution were tested: vacuum distillation, steam distillation and distillation-extraction. At ppm and ppb levels, the best recovery and reproducibility were obtained with the distillation-extraction method. A comparison between three concentration methods has demonstrated that the nitrogen evaporation gave the best recovery value with a reproducibility similar to that of the rotovapour. When routine works are involved, the distillation-extraction method seems to be a good compromise to isolate volatile from aqueous products.

Keywords; aroma, isolation, distillation, model solution, recovery

Arome research has developed rapidly since the introduction of advanced analytical instrumentation, such as gas chromatography and gas chromatography-mass spectrometry. However, the composition and the quantitation of the aroma, in a particular sample, can vary tremendously according to the isolation and concentration method used (HEATH & REINECCIUS, 1986).

Numerous methods to isolate and concentrate volatile flavour compounds have been published (BEMELMANS, 1981; HEATH & REINECCIUS, 1986). Different methods can be used according to the type of samples: for solid and aqueous products, isolation can be made by distillation (MORALES & DUQUE, 1987) or extraction methods (MIHARA et al., 1987). Concentration can be made by distillation or extraction. Other methods, such as head-space (MACKU & JENNINGS, 1987), cold traping (ANDERSEN, 1987) or adsorption traps (BUTTERY et al., 1987), are used to isolate and concentrate the volatiles from the vapour phase.

No single method will provide a flavour profile truly representative of the food (HEATH & REINECCIUS, 1986), particularly when unfamiliar samples are analysed. For the laboratory routine of testing well defined samples, the chosen method must be a compromise between different aspects of the analytical procedure, such as time consumption, accuracy, precision and reproducibility, particularly when quantitative work is involved. The aim of this study is to evaluate three standard methods of isolation and three methods of con-

centration of the samples that can be used in routine aroma analysis. The distillation methods were chosen for the isolation of aroma because they are very popular, simple, and efficient (HEATH & REINECCIUS, 1986). Two levels of concentration were studied because a large range of concentrations (a few ppb to hundreds of ppm) can be found in natural samples.

The steam and vacuum distillations have been frequentely used and are well described in previous literature (BEMELMANS, 1981). The distillation-extraction methodology, such as the Likens–Nickerson apparatus, is a method in which simultaneous steam distillation and extractive processes occur. However, considering thef requent application of this system in the field of flavour isolation, surprisingly little is known about the recovery that is achieved (BEMELMANS, 1981). The main disadvantage of this apparatus is certainly the potential formation of artefacts when used at atmospheric pressure because products can be exposed to high temperatures. The possible transformation and formation of aldehydes have been observed in some samples (CLARK & NURSTEN, 1977; MCGILL & HARDY, 1977). The artefact formation can be minimized if the apparatus is operated at low pressure (BUTTERY et al., 1976; FLATH & FORREY, 1977). In spite of these inconveniences, the distillation-extraction method has been widely used for all types of samples (BEMELMANS, 1981; VERNIN et al., 1985; HORVAT & SENTER, 1985).

1. Materials and methods

Because our research works are dealing with blueberry flavour, the major components of the blueberry flavour were used in the test model solution to evaluate the methods. From a stock solution containing equal quantities of all the volatiles dissolved in methanol, the concentrated solutions (2.17 ppm) were prepared by introducing the appropriate volume directly in the Super-Q water (Millipore Co. Canada) using micro-syringe (Hamilton Co. USA). The dilute solution (0.027 ppm) was prepared from the same stock solution but using different dilution factors. The list of the tested compounds is presented in Table 1. They include a large variety of chemical compounds, having from 5 to 10 carbon atoms and different oxygen containing functional groups, that represent a typical natural sample. They are listed in the elution sequence from the gas chromatograph.

Three extraction procedures were used for the tests: distillation-extraction (Likens–Nickerson apparatus), vacuum distillation, and steam distillation.

The Likens–Nickerson apparatus was constructed as described in MAARSE and KEPNER (1970). The methodology described by HORVAT and SENTER (1985) was followed, except that 120 cm^3 of pentane (Caledon Co., Canada) were used for the extraction.

Table 1

List of the volatile flavour compounds used for the recovery study

Number	Compound name	Minimum purity (%)
1	Ethyl-2-methylbutyrate	97
2	Ethyl isovalerate	98
3	Hexanal	99
4	Isoamyl acetate	99
5	1-Penten-3-ol	99
6	(R)(+)Limonene	97
7	3-Methyl butan-1-ol	99
8	trans-2-Hexenal	99
9	Ethyl caproate	99
10	Hexyl acetate	99
11	Hexane-1-ol	99
12	cis-3-Hexanol	98
13	trans-2-Hexen-1-ol	97
14	Ethyl caprylate	99
15	(±)Linalool	99
16	a-Terpineol	98
17	(R)(+)-β-Citronellol	98
18	Methyl salicylate	99
19	Nerol	97
20	Geraniol	98
21	2-Ethylphenol	99
22	Eugenol	99

All the chemical compounds used during the experiments were bought from Aldrich Chemical Corporation, USA.

The vacuum distillation was performed as described in PARLIMENT and KOLOR (1975) using pentane as the extraction solvent. The steam distillation apparatus was similar to the one described in HACHEY and SIMARD (1987), except that pentane was used as recovery solvent instead of dimethyl ether.

At the high concentration level (2.17 ppm), the samples (in pentane) were injected directly into the gas chromatograph. At the low concentration level (0.027 ppm), three techniques for the concentration of the volatile compounds were tested for each extraction procedure: nitrogen evaporation (N-Vap, Pierce Co. USA), Kuderna–Danish apparatus (Kontes Ltd. USA) and roto-vapor (Bushnï).

Nitrogen evaporation was done at room temperature (23 °C) and at a flow of purified nitrogen of 6.3 cm^3 per min. Time intervals of 4 to 5 days were necessary to evaporate the solvent down to a volume of 0.5 cm^3. The sample was not dried because important losses of material could occur.

The Kuderna–Danish apparatus was maintained in a water bath at a temperature of 40 °C and a period of 2 to 3 weeks was necessary to bring the initial volume down to 0.5 cm^3. The sample was not dried.

The roto-vapor technique necessitates a control of the vacuum level. To avoid the loss of material, the vacuum was maintained at a level of 10.67 kPa

with a control valve. The temperature of the extract was maintained at −17 to −20 °C by placing the round bottom flask in an ice bath containing $CaCl_2$. The procedure took 4 h. The sample was initially placed in a 250 cm^3 round-bottom flask, then transferred in a 20 cm^3 vial to a final volume of 2.0 cm^3. The sample was cooled in an ice bath during five minutes and then placed under vacuum.

The chromatograph used was a Hewlett–Packard, model 5890, equipped with capillary facilities, an on-column injection system (4 PSI and on-column vent 55 cm min^{-1}) and a flame ionisation detector. The capillary column was a 30 m Supelcowax, with a 0.25 mm i.d. and a coating thickness of 0.25 μm (Supelco Co., USA). The temperature program was as follows: an initial temperature of 35 °C during 6 min; then the temperature was increased to 230 °C

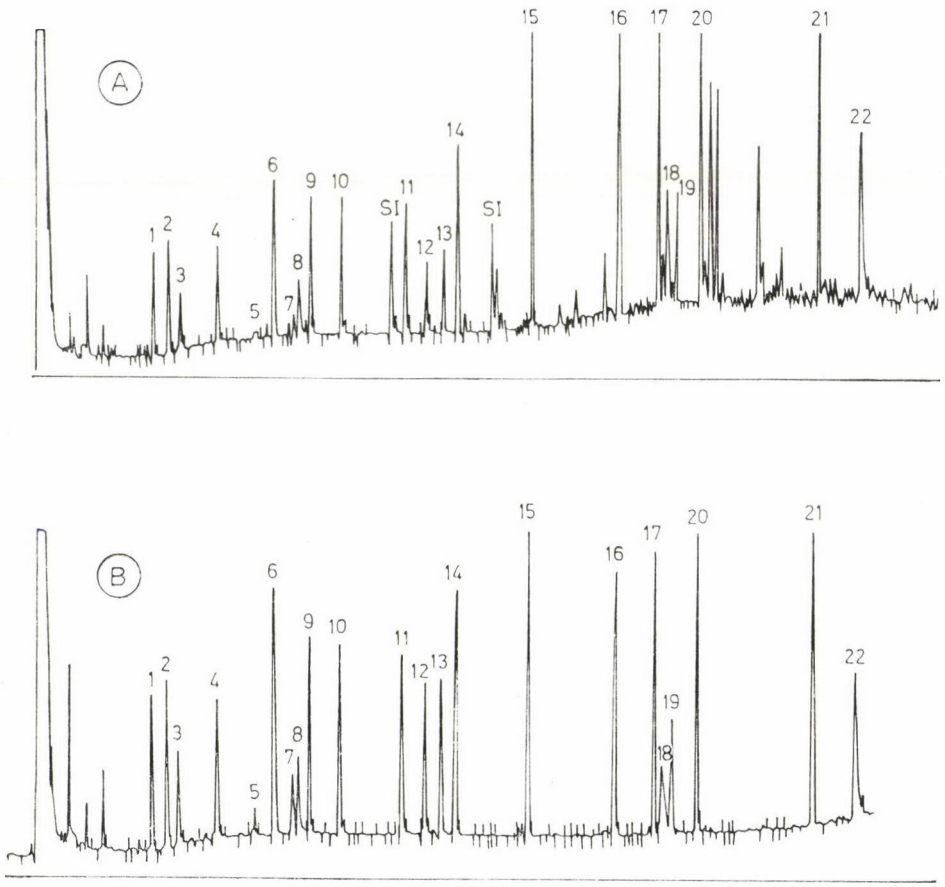


Fig. 1. Chromatograms by a model solution containing 0.027 mg l^{-1} of each of the 22 volatile flavour compounds using steam distillation (A) and distillation-extraction (B). The samples were concentrated using the roto-vapor methodology

at a rate of 5 °C per min; this temperature was maintained during 8 min. The total run time was 51 min. The detector temperature was set at 250 °C. Nitrogen was used as a carrier gas with a linear velocity of 15 cm sec^{-1}. A 10μl syringe (Hamilton Co., USA) and fused silica needle (0.17 mm o. d.) (Chromatographic Specialities Inc., Canada) were used for injection.

An injector temperature of 35 °C was chosen to perform a cool injection as needed to obtain reproducible injections when quantitative analyses are done.

Each compound was identified by its retention time as shown in the two chromatograms presented in Fig. 1.

Quantitation was performed by using the integrated surfaces calculated by a Hewlett–Packard integrator, model 3990A. The computed areas were related to the corresponding concentration using the linear regression equations obtained from the standard calibration curves as shown in Table 2. Each standard curve was constructed with five calibration points within the range of 0 to 100 ppm. Three replicates were necessary to obtaine sufficient statistical data (HARRIS, 1982).

Table 2

Values of the linear regression parameters for the calibration plots of the area values (y) as function of the concentrations (ppm) of individual aroma (x)

Compound number	m ($\times 10^4$)	b ($\times 10^4$)	r	n	S_y (%)
1	7.51	2.84	0.9998	5	4.3
2	8.00	2.15	0.9998	5	4.3
3	6.14	−3.00	0.9998	5	5.0
4	7.78	−2.58	0.9997	5	4.8
5	7.59	−2.01	0.9993	5	5.3
6	12.28	2.37	0.9998	5	4.4
7	8.33	2.92	0.9993	5	4.6
8	7.30	−5.99	0.9999	5	5.1
9	8.35	2.55	0.9998	5	4.6
10	8.37	1.52	0.9998	5	4.5
11	9.67	−0.42	0.9998	5	4.4
12	9.17	−1.65	0.9997	5	4.2
13	8.84	−1.11	0.9998	5	4.5
14	9.09	3.58	0.9998	5	4.6
15	10.62	4.41	0.9999	5	4.5
16	10.26	4.16	0.9999	5	4.5
17	9.87	3.49	0.9999	5	4.5
18	7.07	−6.50	0.9999	5	4.9
19	4.10	1.70	0.9999	5	4.4
20	16.55	8.09	0.9999	5	4.6
21	10.50	0.82	0.9999	5	4.4
22	8.64	−6.45	0.9999	5	5.0

m: slope
b : intercept
r : correlation coefficient
n : number of data points for each standard curve
S_y: percentage of error associated with each linear regression using standard curve triplicates

For all the curves, the correlation coefficients were equal to 1.00, and the relative error associated with each curve was calculated (HARRIS, 1982). The relative error was constant for the range studied. Thus only one error value is presented for each calibration curve. As seen in Table 2, the relative errors vary from $\pm$ 4.2% to $\pm$ 5.3%. An instrumental error of $\pm$5.0% was calculated. Therefore, it seems reasonable to assume that the error associated with the linear regression plot is corresponding to the instrumental error.

2. Results

At ppm concentration levels, the data for individual compounds (mean of 3 essays) are plotted in Fig. 2. The recovery obtained using the distillation--extraction apparatus is better because it recovers all the compounds, independently of their chemical structure, with a very high percentage (97.3%). The data for the recovery of the volatile flavour compounds from the test solution are presented in Table 3. A statistical test of comparison of the means ($P > 0.01$) showed a very significant difference between the three methods. The reproducibility of this method was excellent. The standard deviation on 4 samples was only 1.0%.

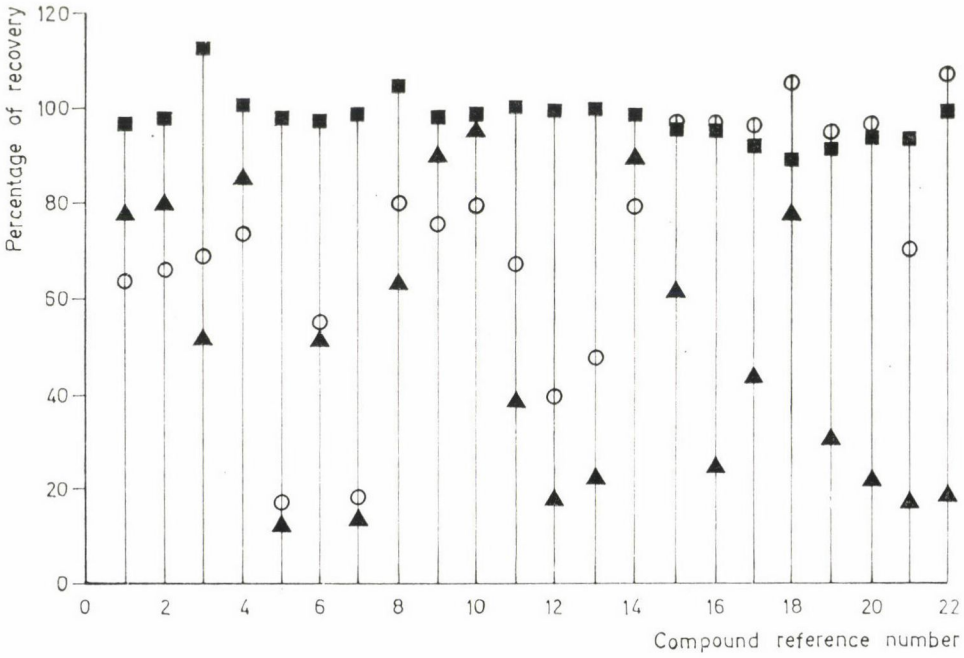


Fig. 2. Percentage of recovery for the concentrate samples containing 2.17 mg l^{-1} of each of the 22 volatile flavour compounds using vacuum distillation (▲), steam distillation (○) and distillation-extraction (■)

Table 3

Average yields of recovery, standard deviation, and range of recovery for each procedure

Method of extraction	Concentration added ($mg\,l^{-1}$)	Mean recovery (%)	Standard deviation (%)	Number of replicates (n)	Range[a] of recovery
Vacuum distillation	2.17	49.7	1.2	3	12.2 – 95.1
Steam distillation	2.17	72.3	3.0	3	16.4 – 107.4
Likens–Nickerson	2.17	97.3	1.0	4	89.4 – 112.1
Vacuum distillation (roto-vapor)	0.027	26.2	2.2	5	2.8 – 52.8
Steam distillation (roto-vapor)	0.027	63.5	10.5	5	7.8 – 125.1
Likens–Nickerson (roto-vapor)	0.027	60.1	6.4	5	19.7 – 70.6
Likens–Nickerson Kuderna–Danish	0.027	64.5	10.5	5	20.4 – 78.2
Likens–Nickerson (N-Vap)	0.027	78.8	7.0	5	24.9 – 91.4

[a] For the 22 compounds used in the experiment, range for the average recovery values.

The three methods of extraction: vacuum distillation, steam distillation, distillation–extraction (Likens–Nickerson apparatus) using a low concentration (0.027 ppm) and a high concentration (2.17 ppm) of volatile flavour compounds. For the low concentration solutions, the extracts were concentrated using one of the three concentration methods: roto-vapor, Kuderna–Danish or Nitrogen-evaporation (N-Vap)

Recoveries between 72 and 97% have been obtained for unspecified hop oils constituents (LIKENS & NICKERSON, 1964). Monoterpene and sesquiterpene fractions of essential oils were recuperated at 97% and 91%, respectively, whereas the thymol fraction was recuperated at 57% only (MAARSE & KEPNER, 1970). It is important to notice the large variability of the isolation for the different compounds with the other methods tested.

At ppb levels, the concentration methods have to be used. They were tested on samples obtained using the distillation-extraction apparatus. The data presented in Table 3 show that the best concentration technique seems to be the N-Vap with a mean recovery of 78.8%, 64.5% for Kuderna–Danish, and 60.1% for roto-vapour. On the other hand, the reproducibility was better using roto-vapour with a standard deviation value of 6.4%. The time involved for the concentration method is very short for the roto-vapour (4 h) compared to the other techniques, N-Vap (4–5 days) and Kuderna–Danish (14–20 days).

However, at ppb concentrations as seen in Fig. 3, the recovery of the distillation-extraction is definitely better, except that the recovery values, whatever the method, are lower than those obtained with the concentrated samples. At low concentrations (ppb) (Table 3), both distillation-extraction and steam distillation seem to be adequate methods, because there is no statistical difference ($P = 0.05$) between the two methods. The percentage of recovery obtained at ppb levels are not as good as those obtained at ppm level

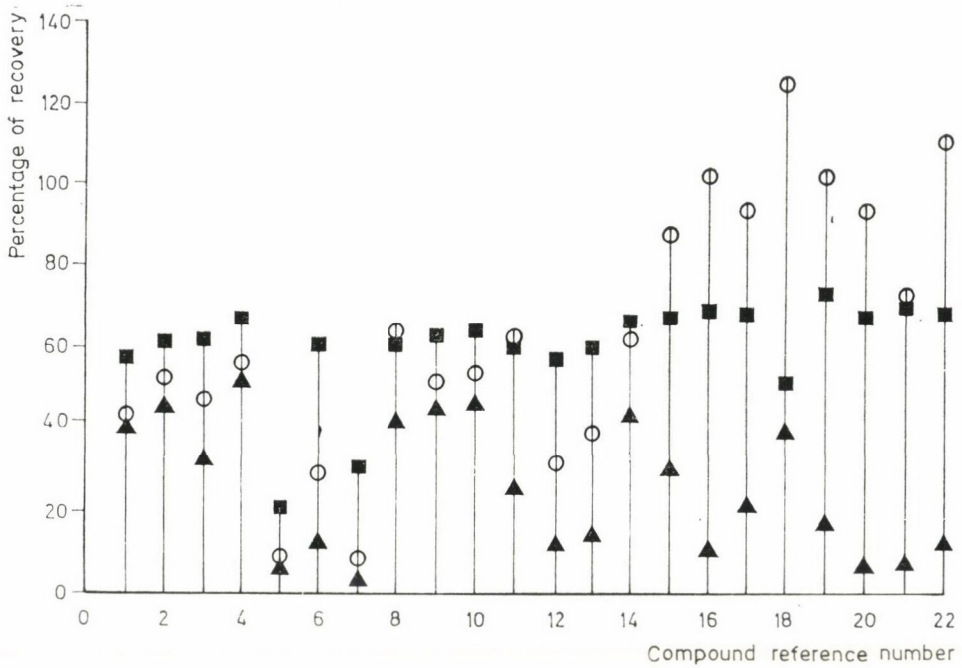


Fig. 3. Percentage of recovery for the diluted samples containing 0.027 mg l^{-1} of each of the 22 volatile flavour compounds using vacuum distillation (▲), steam distillation (○), and distillation-extraction (■). All the samples were concentrated using the rotovapor methodology

because it decreases to only 60.1% for the distillation-extraction apparatus. On the other hand, the standard deviation obtained for the distillation-extraction is only 6.4% compared to 10.5% for the steam distillation, therefore showing a more constant recuperation and a better recovery using the distillation-extraction method.

For two compounds (5 and 7), the yields of recovery are very low in most of the methods used, whatever the concentration. These two compounds are alcohols with 5 carbon atoms and poor recoveries have previously been observed for short chain molecules with five or less carbon atoms whereas the longer ones were almost entirely recovered (using the distillation-extraction apparatus, FARMER et al., 1973). However good data were obtained for these compounds using ether as extraction solvent (SCHULTZ et al., 1977). These data seem to suggest that the same remarks can be made about the two other methods used.

It is interesting to notice the high recovery values above (105%) obtained using the steam distillation method for the compounds # 15 and above. The recovery values above 105% could not be explained by the instrumental error as seen previously. These high values are calculated because the integrations

of the peaks were difficult with the large background level encountered at this level of sensitivity of the chromatograph, in the case of the steam distillation.

In fact, at the ppb level, the concentration step using the roto-vapour concentrates the sample but also many impurities (Fig. 1-A) producing numerous unknown peaks and increasing tremendously the baseline noise from peak 15 and above. Such a background does not appear when using the roto--vapour if the sample has been isolated using vacuum distillation or the distillation-extraction apparatus (Fig. 1-B). The integration mode was the same during all the experiments. The important baseline noise and the appearance of numerous unknown peaks, introduced by the concentrated impurities, produced many deformed peak shapes that increased considerably the integration error, as seen particularly by comparison of peaks 18 and 22 in both Fig. 1-A and 1-B.

The means of recovery and the standard deviations of repetitive tests are helpful to compare different methods and to obtain the level of reproducibility. For a better understanding of the isolation process, it seems pertinent to study the range of recovery obtained for all the compounds studied. The data are presented in Table 3. At high concentrations, the range of extraction is very large for the vacuum and the steam distillation showing a large variability in the yield of extraction of individual compounds. The range of extraction is much smaller for the distillation-extraction process. At ppm

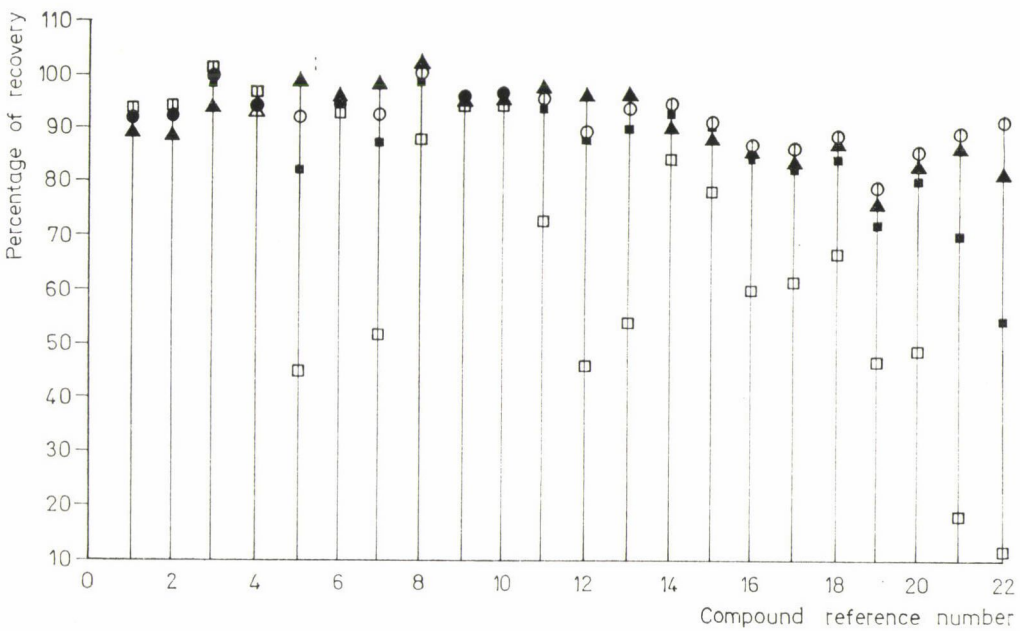


Fig. 4. Percentage of recovery for the concentrated samples containing 2.17 mg l^{-1} of each of the 22 volatile flavour compounds as a function of extraction time, one hour (□), three hours (■), six hours (▲), 12 hours (○) using distillation-extraction

level, this method seems to extract adequately the aroma volatiles. At low concentration, the recovery is very poor for the vacuum distillation and the range is quite large but not as large as the range for steam distillation. The range values of the recoveries obtained by these two methods are large. The best results are definitely obtained with the distillation-extraction apparatus because the range of recovery is smaller than that of using the two other methods, showing a less discriminative process of isolation.

The data presented in Fig. 4 show the percentage of recuperation obtained with the distillation-extraction apparatus for every compounds tested as a function of the extraction time. For extraction times of one and three hours, the percentage of recuperation varies considerably from 12% to 100% depending upon the nature of the compounds. The compounds with low recuperation values have a relatively polar functional group, such as alcohol. After 6 h of extraction, all the tested compounds are adequately recovered with an average value of 97.4 $\pm$ 4.6%. Doubling the extraction time to 12 h does not yield a different average recuperation. The data seem to demonstrate that a 6 h period of extraction is adequate to extract aroma volatiles having quite different functional groups and carbon numbers.

3. Conclusions

The data obtained in this study seem to demonstrate the usefulness of the distillation-extraction method for the recovery of volatile flavour compounds. Effectively, SCHULTZ and co-workers (1977) observed earlier that this distillation-extraction apparatus can be a very efficient flavour extractor but their data were obtained on relatively concentrated samples (165 ppm). The data obtained in this study show that the distillation-extraction method is a very efficient extractor at a few ppm level, and as good or better than other distillation methods at the ppb level.

In this study, when using the high concentrations, compounds 3 and 8 show recovery values greater than 100%, as seen in Fig. 1. These two compounds being aldehydes (hexanal and trans-2-hexenal) may reinforce the previous statement and suggest possible artefacts. In spite of a lower percentage of recovery, vacuum and temperature controlled roto-vapour constitute rapid methods to concentrate the volatiles at all the levels of concentration expected in a natural sample. A good discussion of volatile concentration and possible artefact formation using the distillation method is given by BEMELMANS (1981).

From these data, the distillation-extraction method seems to be a good method when considering yields of recovery, time consumption, and reproducibility. However, recovery studies have to be carefully interpreted when model solutions are used because erroneous results can be generated by volatiles

that may have different solubility, that may be found in different parts of the product at different concentrations, and that may be bound to other chemical components such as proteins and carbohydrates. Further works will study the recovery of aroma volatiles in natural samples using the Likens–Nickerson method.

Literature

ANDERSEN, J. F. (1987): Composition of the flora odor of *Cucurbita maxima* Duchesne (Cucurbitaceae). *J. agric. Fd Chem., 35*, 60–62.

BEMELMANS, J. M. H. (1981): – In: MAARSE, H. & BELZ, R. (Eds.) Isolation, separation, and identification of volatile compounds in aroma research. Academie-Verlag, Berlin, pp. 4–58.

BUTTERY, R. G., GUADAGNI, D. G., LING, L. C., SEIFERT, R. M. & LIPTON, W. (1976): Characterization of additional volatile components of tomato. *J. agric. Fd Chem., 21*, 829–832.

BUTTERY, R. G., LING, L. C. & LIGHT, D. M. (1987): Tomato leaf volatile aroma components. *J. agric. Fd Chem., 35*, 1039–1042.

CLARK, R. G. & NURSTEN, H. E. (1977): Volatile flavour components of brazil nut, *Bertholletia excelsa* (Humb. and Bonpl.). *J. Sci. Fd Agric., 27*, 713–720.

FARMER, J. W., HUME, A. & BURT, J. R. (1973): Efficiency of Likens and Nickerson distillation method for carbon compounds up to C_{11}. *Chem. Ind. (London)*, 279–280.

FLATH, R. A. & FORREY, R. R. (1977): Volatile components of papaya (*Carica papaya* L., Solo variety). *J. agric. Fd Chem., 25*, 103–108.

HACHEY, J. M. & SIMARD, S. (1987): Extraction and analysis of the essential oil of the needles and twigs of white spruce *Picea glauca* (Moench) Voss. *J. Wood Chem. Technol., 7*, 333–341.

HARRIS, D. C. (1982): *Quantitative chemical analysis*. W. H. Freeman and Company, New York, pp. 53–58.

HEATH H. B. & REINECCIUS, G. (1986): *Flavour chemistry and technology*. Van Nostrand Reinhold, New York, pp. 3–36.

HORVAT, R. J. & SENTER, S. D. (1985): Comparison of the volatile constituents from rabbiteye blueberries *(Vaccinium Ashei)* during ripening. *J. Fd Sci., 50*, 429–436.

LIKENS, S. T. & NICKERSON, G. B. (1964): Detection of certain hop constituents in brewing products. *Am. Soc. Brewing Chem. Proc.*, 5–13.

MAARSE, H. & KEPNER, R. E. (1970): Changes in composition of volatile terpenes in Douglas Fir needles during maturation. *J. agric. Fd Chem., 18*, 1095–1101.

MACKU, C. & JENNINGS, W. G. (1987): Production of volatiles by ripening bananas. *J. agric. Fd Chem., 35*, 845–848.

MCGILL, A. S. & HARDY, R. (1977): Artefact production in the distillation-extraction apparatus when used to extract the volatile flavorous components of cod. *J. Sci. Fd Agric., 28*, 89–92.

MIHARA, S., TATEBA, H., NISHIMURA, O., MACHII, Y. & KISHINO, K. (1987): Volatile components of chinese quince (*Pseudocydonia sinensis* Schneid). *J. agric. Fd Chem., 35*, 532–537.

MORALES, A. L. & DUQUE, C. (1987): Aroma constituents of the fruit of the mountain papaya *(Caraca pubescens)* from Colombia. *J. agric. Fd Chem., 35*, 538–540.

PARLIMENT, T. H. & KOLOR, M. B. (1975): Identification of the major volatile components of blueberry. *J. Fd Sci., 40*, 762–763.

SCHULTZ, T. H., FLATH, R. A., MON, T. R., EGGLING, S. B. & TERANISHI, R. (1977): Isolation of volatile components from a model system. *J. Fd Chem., 25*, 446–449.

VERNIN, G., METZGER, J., SUON, K. N. & FRAYSSE, D. (1985): Arôme de mirabelle: étude par cg-sm-banque specma. *Parfum. Cosmét. Arômes, 62*, 69–74.

Acta Alimentaria, Vol. 18 (3), pp. 295—303 (1989)

DIETARY OLIVE OIL AND LIVER CHOLESTEROL IN RATS

A. C. BEYNEN[a, b]

[a]Department of Laboratory Animal Science, State University, P. O. Box 80.166, 3508 TD Utrecht. The Netherlands
[b]Department of Human Nutrition, Agricultural University, P. O. Box 8129, 6700 EV Wageningen. The Netherlands

(Received: 1 August 1988, accepted: 14 December 1988)

Rats were fed semipurified diets containing 1% (w/w) of cholesterol and 10% of fat consisting of various mixtures of coconut fat and olive oil. Substitution of olive oil for coconut fat caused significant increases in serum and liver cholesterol. These effects were already observed when 2% of coconut fat was replaced by olive oil. The increase in liver cholesterol ranged from 40 to 47% when up to 8% of coconut fat in the diet was replaced gradually by olive oil. It is concluded that olive oil has a specific liver cholesterol elevating effect in rats.

Keywords; olive oil, coconut fat, serum cholesterol, liver cholesterol, rats

Olive oil is the major edible oil produced in Europe, and its worldwide production is 1624×10^3 metric tons (CONSIDINE, 1982). There is recent evidence that diets rich in monounsaturated fatty acids, especially in the form of olive oil, might be of value in the treatment of hypercholesterolemia and prevention of coronary heart disease (GRUNDY, 1986; MENSINK & KATAN, 1987). In various animal studies, however, dietary oleic acid caused increased concentrations of liver cholesterol when compared with either saturated or polyunsaturated fatty acids (BEYNEN, 1988). It is not known whether high concentrations of liver cholesterol are harmful and whether the animal data can be extrapolated to man. Nevertheless, the possibility that monounsaturated fatty acids increase liver cholesterol in humans should be considered. The increase in liver cholesterol induced by olive oil has been observed in animals fed diets containing olive oil essentially as the sole source of fat (BEYNEN, 1988). Such a situation will not readily occur in man. It could be suggested that olive oil present in the diet at relatively low concentrations would not affect liver cholesterol. In the present study this suggestion was tested using rats.

1. Materials and methods

Female rats of an outbred Wistar colony (Cpb/WU) were used. The animals were housed individually exactly as described earlier (BEYNEN, 1987). At the age of 5 weeks, the rats were divided into 6 dietary groups of 6 animals

each. The groups had similar distributions of serum cholesterol and body weight; the mean values were 3.14 mmol l^{-1} and 127 g.

The basal diet containing 10% (w/w) of coconut fat consisted of (g per 100 g): casein, 21; corn oil, 2; coconut fat, 10; cholesterol, 1; sucrose, 20; corn starch, 36; cellulose, 2; dicalcium phosphate, 2.9; sodium chloride, 0.6; magnesium carbonate, 0.3; magnesium oxide, 0.2; potassium bicarbonate, 1.8; vitamin premix, 1.2 and mineral premix, 1.0. The composition of the vitamin and mineral premix has been described (BEYNEN et al., 1986). Olive oil was added to the diet at concentrations of 2, 4, 6, 8 or 10% (w/w), and was substituted for coconut fat. The analysed fatty acid compositions of corn oil, coconut fat and olive oil were as follows (g per 100 g fatty acids): saturated fatty acids, 14.5, 91.4 and 15.0; monounsaturated fatty acids, 28.6, 6.5 and 76.6; polyunsaturated fatty acids, 56.8, 2.1 and 7.8. The diets were in meal form. Food and water were provided ad libitum. The experiment lasted 24 days.

Blood samples were taken in the non-fasting state by orbital puncture under light diethyl-ether anesthesia between 8.00 and 10.00 h. Serum total cholesterol was measured enzymatically using the kit (Monotest^R) purchased from Boehringer-Mannheim GmbH (Mannheim, F. R. G.). At the end of the experiment, the anesthetized rats were killed by decapitation and the livers removed. Liver cholesterol was extracted and analysed according to ABELL and co-workers (1952).

2. Results

Replacement of dietary coconut fat by olive oil did not significantly affect body weight, liver weight and feed intake of the rats (Table 1).

Table 1

Effect of substitution of olive oil for coconut fat on performance and cholesterol in serum and liver of rats

Dietary fat	Body weight (g)		Feed intake (g per day)		Liver weight (g)		Serum cholesterol (mmol l^{-1})		Liver cholesterol (μmol g^{-1})	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
10% C	188	26	13.8	2.0	9.7	1.7	4.6	1.2	31.8	9.2
8% C + 2% O	179	22	13.5	1.4	9.2	1.8	6.0	1.6*	44.4	28.4
6% C + 4% O	185	12	13.4	0.8	9.5	0.8	7.7	2.0*	53.7	16.3*
4% C + 6% O	171	15	12.6	1.6	8.7	1.3	8.0	2.0*	57.9	23.4*
2% C + 8% O	195	19	14.5	1.6	10.6	1.4	13.2	1.5*	78.6	28.4*
10% O	181	32	13.4	2.2	9.5	2.0	10.9	1.5*	72.5	18.8*

The diets contained various mixtures of coconut fat (C) and olive oil (O), the amounts being expressed as %, w/w. Results are given as means ($\bar{x}$) ± s for 6 rats per dietary group. Versus animals fed coconut fat as sole source of fat: * Significant at $P \leq 0.05$ probability level. (Student's two-tailed t test)

The addition of olive oil to the diet caused a marked increase in both serum and liver cholesterol concentrations. The olive oil effect on serum cholesterol already reached statistical significance at a concentration of 2% of the diet. The increase in liver cholesterol was significant at dietary levels of 4% and higher. The influence of olive oil on serum and liver cholesterol tended to be dose-dependent.

3. Conclusions

This study shows that substitution of olive oil for coconut fat caused an increase in serum and liver cholesterol in rats. The serum cholesterol elevating effect of olive oil has been observed earlier using diets containing 1% of cholesterol and 10% of olive oil versus either coconut fat or corn oil (BEYNEN, 1987). No such effect of olive oil was seen when cholesterol-free diets were used (BEYNEN, 1987). The important finding in the present study is that concentrations as low as 2% of olive oil in a diet containing cholesterol have hypercholesterolemic activity.

The feeding of olive oil resulted in markedly increased liver cholesterol concentrations. This corroborates other studies as reviewed earlier (BEYNEN, 1988). It is clear from the data in Table 1 that olive oil exerts its liver cholesterol elevating effect at relatively low dietary concentrations. This suggests that olive oil has a specific hepatocholesterolic effect in rats. In general, polyunsaturated fatty acids produce higher liver cholesterol concentrations in rats than saturated fatty acids do (J. N. ZSINKA et al., 1988; BEYNEN, 1987). However, dietary monounsaturated fatty acids even induce higher concentrations (BEYNEN, 1988), and this effect is seen at low amounts in the diet (Table 1).

Literature

ABELL, L. L., LEVY, B. B., BRODY, B. B. & KENDALL, F. E. (1952): A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem., 195*, 357–366.

BEYNEN, A. C. (1987): Serum and liver cholesterol in rats fed cholesterol-free or high-cholesterol diets differing in type and amount of fat. *Nutr. Rep. int., 35*, 1327–1332.

BEYNEN, A. C. (1988): Dietary monounsaturated fatty acids and liver cholesterol. *Artery, 15*, 170–175.

BEYNEN, A. C., WEST, C. E., VAN ZUTPHEN, L. F. M. & KATAN, M. B. (1986): Relation between the responses of serum cholesterol to dietary cholesterol and to the type of dietary fat in random-bred rabbits. *Nutr. Rep. int., 33*, 71–78.

CONSIDINE, D. M. (Ed.) (1982): *Foods and food production encyclopedia*. Van Nostrand Reinhold Company, New York.

GRUNDY, S. M. (1986): Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. *New Engl. J. Med., 314*, 745–748.

J. N. ZSINKA, Á., FÖLDES, V. & PERÉDI, J. (1988): Indices of lipid metabolism disturbance induced by diet (Rat experiments). *Acta Alimentaria, 17*, 43–51.

MENSINK, R. P. & KATAN, M. B. (1987): Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. *Lancet, 1*, 122–125.

Acta Alimentaria, Vol. 18 (3), pp. 299—311 (1989)

EFFECT OF EXTRUSION TEMPERATURE ON PHYSICO-CHEMICAL PROPERTIES AND BIOLOGICAL VALUE OF SOYBEAN-PROTEIN

E. Horváth, J. Petres, É. Gelencsér and B. Czukor

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary

(Received: 15 August 1988; accepted: 9 November 1988)

Three extrusion temperatures (120, 160 and 200 °C) were applied as a treatment to evaluate their effects on the physico-chemical properties and biological value of full fat soybean flour, extruded in a Brabender extruder. Water absorption capacity increased, fat absorption capacity, emulgation properties and Nitrogen Solubility Index of soy decreased as extrusion temperature increased.

The extractability of proteins by the methods of Osborne and Than and Shibasaki, and the quantity of water and NaCl soluble proteins decreased with increasing temperature. The buffer containing sodium-dodecylsulphate and 2--mercapto-ethanol was needed to disrupt bonds formed during extrusion. The SH and SS group contents in extracted protein fractions of extruded soy samples and the gelelectrophoretograms showed that extracted protein fractions were affected by extrusion temperature. New protein bands mostly of smaller molecular weight appeared. As shown by the results trypsin inhibitor and urease activity, the in vitro protein digestibility decreased, in vivo biological value increased with increasing extrusion temperature. The highest extrusion temperature (200 °C) has caused the decrease of biological value.

Keywords; Full fat soy flour, extrusion temperature, functional properties, extraction of soy proteins, biological value

In processes of industrial technology different raw materials of plant and animal origin are exposed to different physical and chemical treatments to get high quality products for human consumption and animal feeding. Extrusion processing has become also a well established technology with a number of food and feed applications. It allows the production of processed products, which are similar in certain physical characteristics to products obtained through the use of more costly equipments. In addition to the usual benefits of heat processing, extrusion offers the possibility of modifying the functional properties, the protein structure, decreasing the quantity of antinutritive materials and increasing the biological value (Cheftel, 1986; Cumming et al., 1972; Maurice et al., 1976).

Nowadays changes during food processing are taken into consideration and processing technology can be characterized by full knowledge of results and unfavourable phenomena can be stopped. Final aim is to develop processing technology (e.g. extrusion-cooking) enabling the production of products of high nutritive value without antinutritive materials.

The aim of this study was to establish the effects of increasing extrusion temperature under constant pressure and accelerated screw rate upon the chemical, physical and technological properties and biological value of soybeans of the same moisture content. The reasons why soybean was chosen as a model material are the following: the importance of soybean as a food ingredient is rapidly increasing and it is rich in protein and antinutritive materials.

A number of studies have been published on soya extrusion but almost all investigations of soya extrusion have been confined to defatted soya products: grits, flours, concentrates, isolates (MAURICE et al., 1976; HAGER, 1984; SHEARD et al., 1984, 1985, 1986a, 1986b; SIMONSKY & STANLEY, 1982).

Only some works evaluated the effects of extrusion on the quality of whole soybean and full fat soy flour (MUSTAKAS et al., 1970; BOOKWALTER et al., 1971; BURGESS & STANLEY, 1976; LAM-SÁNCHEZ et al., 1985). Nevertheless full fat soybean can also be taken into consideration as an economical and widely available source of nutritive components.

The purpose of this article is to investigate how different extrusion temperatures affect properties of extruded full fat soy flour, what kind of changes are caused in soy flours as compared to one an other and to raw soy flour. Emphasis will be placed on functional properties, quantity of antinutritive materials, biological value and protein solubility and structure.

1. Materials and methods

1.1. Soy samples

Soybean seeds (Glycine max. var. Ewans) were dehulled. The dehulled soybeans were extruded in a Brabender laboratory extruder, type 20DN (Brabender OHG, Duisburg, GFR) at a constant screw rate of 120 r.p.m. (compression ratio 4 : 1). The diameter of die was 3 mm.

The moisture content of each soy sample was adjusted to 16% (w/w) prior to extrusion.

A temperature range of 120–200 °C was used in three steps to produce a wide range of extruded full fat soy flours. Temperature setting of the three heating zones can be seen in Table 1.

Table 1

Temperatures of extrusion zones applied in the extrusion of full fat soybean flour

Sample	Temperature of extrusion zones (°C)		
	Zone I	Zone II	Zone III
Extruded soy flour 1	40	80	120
Extruded soy flour 2	80	120	160
Extruded soy flour 3	120	160	200

Dehulled soybean and extruded soy samples were ground in a laboratory mill to flour (particle size $\leq$ 600 μm). Extruded samples were collected, placed in plastic bags and stored at 5 °C until required.

1.2. Determination of moisture, protein and lipid contents

Crude protein (N$\times$6.25) content was determined by the Kjeldahl procedure, in an automatic Kjell-Foss equipment.

Moisture content was measured by drying the samples to constant weight at 105 °C.

Lipid content was determined by petroleum ether extraction in a Soxhlet apparatus.

1.3. Determination of functional properties

Water absorption capacity was determined by the method of SOSULSKI (1962), fat absorption capacity by the method of LIN and co-workers (1974), emulsifying activity and emulsion stability by the method of YASUMATSU and co-workers (1972), Nitrogen Solubility Index (NSI) by the method of SMITH and CIRCLE (1972).

1.4. Extraction and fractionation of soy proteins

The fat free soy samples were extracted according to the method of OSBORNE (1924), and to the method of THAN and SHIBASAKI (1976). The fat free soy samples were also extracted by 0.5 mol dm^{-3} Na_2HPO_4 (pH = 7.8) buffer, containing 5% (w/v) sodiumdodecyl sulphate (SDS) and 1% (v/v) 2-mercaptoethanol (2-ME).

1.5. Determination of SH and SS groups

The quantity of SH and SS groups in proteins was determined according to the method of BEVERIDGE and co-workers (1974).

1.6. Determination of trypsin inhibitor and urease activities

The trypsin inhibitor activity was determined according to KAKADE and co-workers (1974), as adapted by PETRES and KÁRPÁTI (1981).

The urease activity was determined according to HUNGARIAN STANDARD (1981). Fat free soy samples were used for both determinations.

1.7. Determination of saccharose, raffinose and stachyose contents

Saccharose, raffinose and stachyose contents were measured by the method of SENKÁLSZKY-ÁKOS and co-workers (1984).

1.8. Determination of in vitro digestibility

In vitro protein digestibility was measured by the method of AKESON and STAHMAN (1964).

1.9. Determination of in vivo biological values

The in vivo biological values were measured by feeding tests of growing male rats (Spraque Dowley) by methods of PELLETT and YOUNG (1980).

After a four days adjustment period the test period lasted ten days. 10 rats were assigned to each test group and to a non-protein diet group. The animals were fed and provided with water ad libitum. Fecal matter was collected during the test period.

Net Protein Ratio (NPR) and Net Protein Utilization (NPU) were determined by direct measurement of change in body mass and body nitrogen per unit nitrogen consumption. The Biological Value (BV) and True Digestibility (TD) were calculated from the data of NPU experiments. Nitrogen content of feed, total body and faeces was analyzed by the Kjeldahl method.

1.10. Polyacrylaminde gel electrophoresis (PAGE)

SDS-PAGE was performed on vertical 1 mm slab gels (11.5% (w/v) polyacrylamide) according to the method of WEBER and OSBORN (1975). Gels were stained and destained by the method of STECK and co-workers (1980).

2. Results and discussion

2.1. Moisture, protein and lipid contents of soy samples

Table 2 shows the effects caused by different temperature treatments on the moisture, protein and lipid contents of extruded soy samples. The moisture content of extruded soy samples wetted prior to extrusion decreased with the increase of extrusion temperature. The protein ($N \times 6.25$) and lipid contents of samples are given referring to dry matter. The lipid content of soy flour 3 (200 °C) decreased because some free oil was expelled from soybean during extrusion at 200 °C. The protein content of samples practically did not change. Our results agree with those obtained by other workers (LAM-SANCHEZ et al., 1985).

2.2. Functional properties

The effect of different extrusion temperatures on functional properties of full fat soy flour is shown in Table 3.

Table 2

Moisture, lipid and protein (N×6.25) content of dehulled soybean and its extruded flours

Sample	Moisture content (g per 100 g total weight)		Lipid content (g per 100 g dry weight)		Protein (N×6.25)	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Dehulled soybean	9.7	0.36	25.6	0.1	39.1	0.1
Extruded soy flour 1 (120 °C)	10.1	0.01	24.7	0.2	38.8	0.1
Extruded soy flour 2 (160 °C)	8.5	0.02	24.0	0.2	37.7	0.1
Extruded soy flour 3 (200 °C)	7.7	0.36	21.3	0.1	40.7	0.1

$\bar{x}$: mean value; ±s: standard deviation; n = 3

Table 3

Functional properties of dehulled soybean and its extruded flours

Sample	Water absorption capacity (g per 100 g sample)		Fat absorption capacity		Emulsifying capacity (%, v/v)		Emulsion stability (%, v/v)		Nitrogen Solubility Index (NSI) (%, w/w)	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Dehulled soybean	154.8	2.6	111.0	4.9	76.4	2.1	66.6	2.4	77.5	4.3
Extruded soy flour 1 (120 °C)	200.1	5.7	104.7	6.2	66.8	1.3	71.8	2.1	62.2	1.4
Extruded soy flour 2 (160 °C)	199.0	5.7	95.8	4.4	56.4	0.6	53.8	0.9	15.5	0.6
Extruded soy flour 3 (200 °C)	167.1	6.4	88.6	6.1	56.1	0.5	52.4	0.8	10.2	0.2

For legends see Table 2

Data reveal the effect of extrusion temperature on functional properties. Extruded soy flour samples have higher water absorption capacity (WAC) than dehulled control soy sample. Soy flours 1 and 2 extruded at temperature 120 °C and 160 °C respectively, have the same WAC. Upon increasing the extrusion temperature to 200 °C the WAC of soy sample decreased. CUMMING and co-workers (1972) had also shown that rehydration ability of extruded defatted soybean meal was strongly related to extrusion temperature. It was found that the increase in water uptake with increasing process temperature is largely a result of changes in density. According to MAURICE and co-workers (1976) two structural changes occur in defatted soy meal as extrusion tempera-

ture increases: an increase in aligned fibers reflected in enhanced cohesiveness and the development of a spongy structure as evidence by an increase in porosity.

Work of NOGUCHI and co-workers (1981) showed that WAC of rice extrudate was equally contributed to by the soluble and insoluble carbohydrate components. Addition of soy protein isolate to the rice flour increased its ultrastructural clarity but did not contribute to the WAC. WAC is the outcome not only of gelatinized starch but equally of the insoluble component. This result suggests that protein does not contribute to water absorption capacity.

The effect of extrusion temperature on fat absorption capacity (FAC), emulsifying activity (EA) and emulsion stability (ES) of full fat soy flour was not studied by others. We found that FAC of extruded samples decreased with the increase of temperature. Values EA and ES practically decreased also with increasing temperature up to 160 °C. Extruded soy flours 2 and 3 (160° and 200 °C) have the same EA and ES.

Nitrogen Solubility Index (NSI) values of extruded soy flours decreased with increased extrusion temperature. The greatest change was noticed when the die temperature increased from 120 °C to 160 °C. In that case the NSI value decreased from 62.2% to 15.5%. The decrease of NSI agrees with the results obtained by other workers (MUSTAKAS et al., 1970; BOOKWALTER et al., 1971). CUMMING and co-workers (1973) have also shown that extrusion causes a dissociation followed by an aggregation of the water-soluble proteins.

2.3. Extraction and fractionation of soy proteins

Solubility of soy proteins of different extruded full fat soy flours was examined by using at first the Osborne method. The results are shown in Table 4.

Extrusion decreased the quantity of proteins soluble by this method. Increasing the extrusion temperature reduced the percentage of extracted proteins in total protein. Only 11.5% of proteins could be extracted from extruded soy flour 3 (200 °C). The proportions of individual fractions in total proteins had also changed after extrusion. Upon increasing the temperature the proportion of water and NaCl soluble proteins progressively decreased, denoting denaturation not only of albumin but also of globulin. Albumin and globulin are sensitive to heat denaturation.

Different works of SHEARD and co-workers (1984, 1985, 1986a, b) showed that extrusion produces large aggregates of soy proteins that cause the texturized product to be insoluble. BURGESS and STANLEY (1976) selected three reagents, 2-ME, urea and SDS to characterized extraction of texturized soy. Buffered urea dissolves denatured molecules and small aggregates. SDS disrupts noncovalent interactions and 2-ME is an agent to reduce disulfide bonds.

Table 4

Extraction of proteins from dehulled soybean and its extruded soy flours by Osborne-method

Samples	Extracted proteins in total protein (%, w/w)		Protein fractions in total protein (%, w/w)							
			Water-soluble		NaCl-soluble		C_2H_5OH-soluble proteins		KOH-soluble	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Dehulled soybean	70.6	5.2	33.9	3.8	28.7	2.0	2.8	0.2	5.3	2.4
Extruded soy flour 1 (120 °C)	42.6	6.9	10.8	4.1	24.1	3.7	3.1	1.1	4.6	4.3
Extruded soy flour 2 (160 °C)	19.9	0.8	4.5	0.3	8.3	0.9	1.2	0.3	5.8	0.8
Extruded soy flour 3 (200 °C)	11.5	0.3	3.8	1.6	4.1	1.3	1.2	0.4	2.4	1.2

For legends see Table 2
n = 4

Table 5

Extraction of proteins from dehulled soybean and its extruded soy flours by 0.5 mol dm^{-3} Na_2HPO_4 solution (pH = 7.8) containing 5% SDS and 1% 2-ME

Sample	Extracted proteins in total protein (%, w/w)		Protein fractions in extracted protein (%, w/w)			
			1st extraction		2nd extraction	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Dehulled soybean	42.5	4.4	57.9	8.6	42.1	8.6
Extruded soy flour 1 (120 °C)	78.4	1.5	76.8	0.5	23.2	0.5
Extruded soy flour 2 (160 °C)	58.3	2.3	59.3	0.1	40.7	0.1
Extruded soy flour 3 (200 °C)	54.2	1.7	72.2	0.7	27.8	0.7

For legends see Table 2
(n = 4)

We used 0.5 mol dm^{-3} Na_2HPO_4 (pH = 7.8) buffer containing 5% SDS and 1% 2-ME to extract soy proteins. The results can be seen in Table 5.

The buffer extracted more proteins from extruded flours than from dehulled soybeans. The highest percentage protein was extracted from soy flour 1 (120 °C). Increasing the extrusion temperature up to 160 °C the percentage of extracted proteins decreased and increasing the temperature up to 200 °C only a small increase in percentage of extracted proteins could be noticed.

Our results suggest that low-temperature (120 °C) extrusion forms structured protein primarily by intermolecular disulfide bridging accompanied by changes in noncovalent bonding. In extrusion at higher temperatures (160 °C, 200 °C) other covalent linkages and protein polimerization through formation of intermolecular peptide bonds may also be involved. These results agree with those reported by HAGER (1984).

The proteins were extracted from extruded soy samples by the method of THAN and SHIBASAKI (1976), as well. The results are shown in Table 6.

Table 6

Extraction of proteins from soy samples by the method of Than and Shibasaki

Sample	Extracted protein in total protein (%, w/w)		Protein fractions in total protein (%, w/w)					
			7S protein		11S protein		Whey protein	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Dehulled soybean	90.3	1.2	39.6	3.4	10.7	0.8	40.0	1.2
Extruded soy flour 1 (120 °C)	97.2	1.3	47.7	4.1	12.8	1.6	36.6	8.8
Extruded soy flour 2 (160 °C)	60.8	1.1	24.2	0.4	6.9	0.2	29.7	1.1
Extruded soy flour 3 (200 °C)	48.1	0.8	14.4	0.3	4.4	0.1	29.2	1.7

For legends see Table 2

When the extrusion temperature was raised to 160 °C or 200 °C the amount of protein extracted was lower by this method, too, than in the control sample and in the sample extruded at 120 °C.

2.4. SH and SS groups of protein fractions

The quantity of available SH and total SS groups in lyophilized protein fractions of soy samples is shown in Tables 7 and 8.

Soy protein fractions extracted from different extruded soy samples have not the same SH and SS group contents. No conclusion can be drawn, however, from the results as to the effect of extrusion temperature upon the SH and SS groups contents of the different protein fractions.

2.5. Electrophoresis

The soluble extracts from soybean and the extruded flours were submitted to electrophoresis by SDS-PAGE. Figures 1 and 2 show water, NaCl and KOH soluble protein fractions of soybean samples.

Table 7

Available SH group content of protein fractions extracted from soy samples (μmol g^{-1} fraction)

Protein fraction	Soy samples							
	Dehulled soybean		Extruded soy 1 (120 °C)		Extruded soy 2 (160 °C)		Extruded soy 3 (200 °C)	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
7S protein	10 2	0.4	7.0	0.4	4.8	0.3	12.2	0.5
11S protein	5.5	0.3	11.3	0.6	7.8	0.1	9.0	0.2
Whey protein	6.3	0.2	17.0	0.3	13.1	0.2	15.8	2.1
H_2O-soluble	12.5	0.4	9.5	0.4	9.4	0.3	5.9	0.2
NaCl-soluble	7.8	0.4	7.0	0.3	6.1	0.1	5.3	0.3
C_2H_5OH-soluble	5.0	0.1	6.5	0.3	5.9	0.3	6.4	0.2
KOH-soluble	3.6	0.1	5.0	0.2	4.2	0.4	5.4	0.4

For legends see Table 2

Table 8

SS group content protein fractions extracted from soy samples (μmol g^{-1} fraction)

Protein fraction	Soy samples							
	Dehulled soybean		Extruded soy 1 (120 °C)		Extruded soy 2 (160 °C)		Extruded soy 3 (200 °C)	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
7S protein	44.2	5.1	92.5	3.7	107.3	5.1	107.9	1.9
11S protein	177.7	10.5	97.7	1.9	144.7	4.8	156.9	3.3
Whey protein	64.2	4.1	71.7	1.6	104.4	3.9	102.6	2.8
H_2O-soluble	49.8	4.8	97.1	16.3	60.3	3.8	81.5	10.3
NaCl-soluble	78.2	15.9	72.8	3.5	63.9	2.7	72.9	1.1
KOH-soluble	40.3	2.1	71.1	2.2	64.6	4.2	77.6	0.5

For legends see Table 2

SDS-PAGE revealed that the major subunits of water, NaCl, and KOH soluble proteins of soybean were not affected by extrusion, but new bands of smaller molecular weight proteins appeared, showing degradation of the high molecular weight proteins. Under the effect of extrusion temperature 7S, 11S and whey fractions of soy proteins underwent changes at the subunit level through formation of new bands. NOGUCHI and co-workers (1981) have found that fraction 7S is more affected by extrusion temperature than the fraction 11S. Our results show that the extrusion temperature caused considerable changes in fractions 7S, 11S and whey proteins as well, and revealed another evidence of changes in protein structure during extrusion.

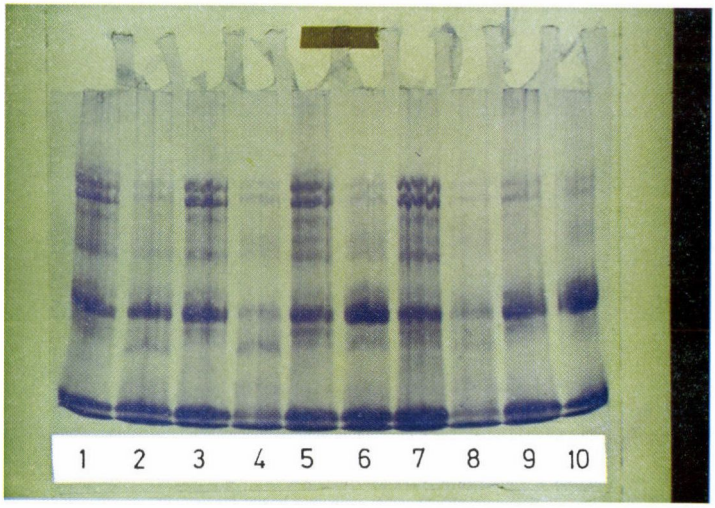


Fig. 1. Protein patterns from dehulled soybean and its extruded soy flours. 1: Water soluble protein (WSP) of dehulled soybean (DS), 2: WSP of extruded soy flour (ESF) 2 (160 °), 3: WSP of ESF 1. (120 °), 4: WSP of ESF 3. (200 °C), 5: NaCl-soluble protein (NSP) of DS, 6: NSP of ESF 2. (160 °C), 7: NSP of ESF 1. (120 °C), 8: NSP of ESF 3. (200 °C), 9: KOH-soluble protein (KSP) of DS, 10: KSP of ESF 2. (160 °C)

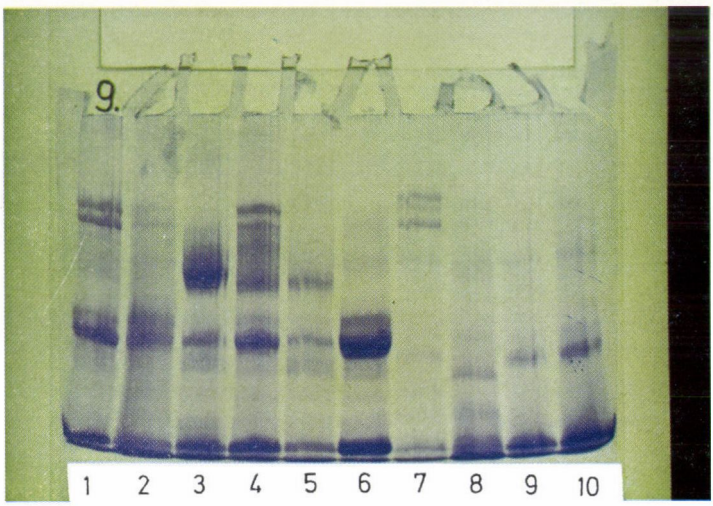


Fig. 2. Protein patterns from dehulled soybean and its extruded soy flours. 1: KSP of ESF 1. (120 °C), 2: KSP of ESF 3. (200 °C), 3: 7S protein of DS, 4: 7S protein of ESF 1. (120 °C), 5: 11S protein of DS, 6: 11S protein of ESF 2. (160 °C), 7: whey protein (WP) of DS, 8: WP of ESF 2. (160 °C), 9–10: WP of ESF 1. (120 °C)

2.6. Trypsin inhibitor and urease activity

Table 9 shows the effect of extrusion temperature on trypsin inhibitor and urease activities of soy samples.

Table 9

Trypsin inhibitor and urease activity of dehulled soybean and its extruded soy flours

Sample	Trypsin inhibitor (TIU per mg defatted sample)		Urease (mg N per g defatted sample per min)	
	x	±s	x	±s
Dehulled soybean	173.2	2.2	10.9	0.1
Extruded soy flour 1 (120 °C)	145.5	3.7	9.2	0.1
Extruded soy flour 2 (160 °C)	27.6	1.1	0.5	0.05
Extruded soy flour 3 (200 °C)	6.8	0.4	0.5	0.05

For legends see Table 2

Values of trypsin inhibitor and urease activities decreased with increased temperature. The urease is more heat labile because its activity at 160 °C is very small and does not change upon increasing the temperature up to 200 °C.

These results agree with the results reported earlier by several authors (MUSTAKAS et al., 1970; BOOKWALTER et al., 1971).

2.7. In vitro and in vivo biological value

In vitro biological value of soy samples was characterized by in vitro protein digestibility (Table 10) and in vivo biological values by NPU, BV, TD and NPR values (Table 11).

In vitro protein digestibility of extruded soy flour 1 (120 °C) was higher than that of dehulled soybean. Increasing the temperature up to 160 °C and 200 °C the in vitro protein digestibility decreased. The results of true protein digestibility (TD) are not in accordance with in vitro digestibilities. Values of NPU and BV show that extruded soy flour 2 (160 °C) has the highest in

Table 10

In vitro protein digestibility of dehulled soybean and its extruded flours

Sample	In vitro protein digestibility (g protein per 100 g)	
	$\bar{x}$	±s
Dehulled soybean	92.4	3.3
Extruded soy flour 1 (120 °C)	95.8	1.3
Extruded soy flour 2 (160 °C)	90.1	0.5
Extruded soy flour 3 (200 °C)	86.3	2.1

For legends see Table 2

Table 11

Biological value of dehulled soybean and its extruded soy flours

Samples	NPU (%)		BV (%)		TD (%)		NPR (g per g N)	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Dehulled soybean	35.6	8.6	29.8	6.6	84.1	1.9	13.9	1.9
Extruded soy flour 1 (120 °C)	48.8	4.4	40.1	4.0	82.3	0.9	19.3	0.9
Extruded soy flour 2 (160 °C)	71.3	5.4	61.5	4.4	86.3	0.3	26.0	0.1
Extruded soy flour 3 (200 °C)	65.2	6.1	56.7	5.6	86.9	0.4	27.9	1.1

$\bar{x}$: means of subgroups; ± s: standard deviation; n (number of subgroups) = 2; number of animals in subgroups = 5

vivo biological value. Extrusion improves the nutritional value of soy protein through thermal unfolding of the major proteins and thermal inactivation of antinutritive factors. At high temperature (200 °C) amino acid losses, Maillard condensation and reduction of protein digestibility can take place (CHEFTEL, 1986).

3. Conclusions

Summarizing all the results it can be seen that functional properties, extraction, structure, antinutritive factors and biological value of soy proteins had changed as an effect of heat treatment during extrusion.

The degree of extrusion temperature also affects the determined properties. It is suggested that one of the main reasons for the changes lies in the modification of soy proteins during extrusion. Our results also proved that the parameters of extrusion conditions were very important in obtaining the needed quality of extruded soybean.

Literature

AKESON, W. R. & STAHMAN, M. A. (1964): A pepsin pancreatin digest index of protein quality evaluation. *J. Nutr., 83,* 257–261.

BEVERIDGE, T., TOMA, S. J. & NAKAI, S. (1974): Determination of SH and SS groups in some food proteins using Ellman's reagent. *J. Fd Sci., 39,* 49–51.

BOOKWALTER, G. N., MUSTAKAS, G. C., KWOLEK, W. F., MCGHEE, J. E., & ALBRECHT, W. J. (1971): Full fat soy flour extrusion cooked: properties and food uses. *J. Fd Sci., 36,* 5–9.

BURGESS, L. D. & STANLEY, D. W. (1976): A possible mechanism for thermal texturization of soybean protein. *Can. Inst. Fd Sci. Technol. J., 9,* 228–231.

CHEFTEL, J. C. (1986): Nutritional effects of extrusion-cooking. *Fd Chem., 20,* 263–283.

CUMMING, D. B., STANLEY, D. W. & DE MAN, J. M. (1972): Texture–structure relationships in texturized soy protein. — Part II. Textural properties and ultrastructure of an extruded soybean product. *Can. Inst. Fd Sci. Technol. J., 5*, 124–128.

CUMMING, D. B., STANLEY, D. W. & DE MAN, J. M. (1973): Fate of water soluble soy protein during thermoplastic extrusion. *J. Fd Sci., 38*, 320.

HAGER, D. F. (1984): Effects of extrusion upon soy concentrate solubility. *J. agric. Fd Chem., 32*, 293–296.

HUNGARIAN STANDARD (1981): Szója ureázaktivitás meghatározása. (Determination of urease activity in soybeans.) MSZ 6830/34–81.

KAKADE, M. L., RACKIS, J. J., MCGHEE, J. E. & PUSKI, G. (1974): Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. *Cereal Chem., 51*, 376–382.

LAM-SANCHEZ, A., BRESSANI, R., MOLINA, M. R., ELIAS, L. G., GONZALES, J. M. & DURIGAN, J. F. (1985): Effects of cone opening, initial moisture content and multiple extrusion on the protein quality of extruded soybean using the Brady Crop cooker. *Archos. lat. am. Nutr., 35*, 447–457.

LIN, M. J. Y., HUMBERT, E. S. & SOSULSKI, F. W. (1974): Certain functional properties of sunflower meal products. *J. Fd Sci., 39*, 368–370.

MAURICE, T. J., BURGESS, L. D. & STANLEY, D. W. (1976): Textures–structure relationships in texturized soy protein. — Part III. Textural evaluation of extruded products. *Can. Inst. Fd Sci. Technol. J., 9*, 173–176.

MUSTAKAS, G. C., ALBRECHT, W. J., BOOKWALTER, G. N., MCGHEE, J. E., KWOLEK, W. F. & GRIFFIN, E. L. (1970): Extruder-processing to improve nutritional quality, flavor and keeping quality of full fat soy flour. *Fd Technol., 24*, 1290–1296.

NOGUCHI, A., KUGIMIYA, W., HAQUE, Z. & SAIO, K. (1981): Physical and chemical characteristics of extruded rice flour and rice flour fortified with soybean protein isolate. *J. Fd Sci., 47*, 240–245.

OSBORNE, T. B. (1924): The vegetable proteins. 2. ed. Longmans, Green Co., London.

PELLETT, P. L. & YOUNG, V. R. (1980): *Nutritional evaluation of protein foods.* United Nations University, p. 150.

PETRES, J. & KÁRPÁTI, GY. (1981): Szójatermékek tripszin inhibitor aktivitásának meghatározása. (Determination of the trypsin inbibitory activity of soy products.) *Élelmiszerv. Közl., 37*, 179–186.

SENKÁLSZKY-ÁKOS, É., PETRES, J. & CZUKOR, B. (1984): Szacharóz-, raffinóz- és sztachióztartalom meghatározása hüvelyesekben. (Determination of saccharose, raffinose and stachyose contents in legumes.) *Élelmiszerv. Közl., 30*, 119–125.

SHEARD, P. R., LEDWARD, D. A. & MITCHELL, J. R. (1984): Role of carbohydrates in soya extrusion. *J. Fd Technol., 19*, 475–483.

SHEARD, P. R., MITCHELL, J. R. & LEDWARD, D. A. (1985): Comparison of the extrusion cooking of a soya isolate and a soya flour. *J. Fd Technol., 20*, 763–771.

SHEARD, P. R., FELLOWS, A., LEDWARD, D. A. & MITCHELL, J. R. (1986a): Macromolecular changes associated with the heat treatment of soya isolate. *J. Fd Technol., 21*, 55–60.

SHEARD, P. R., MITCHELL, J. R. & LEDWARD, D. A. (1986b): Extrusion behaviour of different soya isolates and the effect of particle size. *J. Fd Technol., 21*, 627–641.

SIMONSKY, R. W. & STANLEY, D. W. (1982): Texture–structure relationships in textured soy protein. — Part V. Influence of pH and protein acylation on extrusion texturization. *Can. Inst. Fd Sci. Technol. J., 15*, 294–301.

SMITH, A. K. & CIRCLE, S. J. (1972): *Soybeans chemistry and technology.* O. C. S. Method Ba 11–65. AVI Publishing Company, Inc., Westport, Connecticut, Vol. 1., pp. 451–452.

SOSULSKI, F. W. (1962): The centrifuge method for determining flour absorption in hard red spring wheats. *Cereal Chem., 39*, 344–350.

STECK, G., LEUTHARD, P. & DÜRK, P. R. (1980): Detection of basic proteins and low molecular weight peptides in polyacrylamide gels by formaldehyde fixation. *Anal. Biochem., 107*, 21–24.

THAN, V. H. & SHIBASAKI, K. (1976): Major proteins of soybean seeds. *J. agric. Fd Chem., 24*, 1117–1121.

WEBER, K. & OSBORN, M. (1975): — in: NAURATH, H. & HILL, R. L. (Eds.) *The proteins.* III. ed. Academic Press, N. Y., Vol. 1, pp. 179–223.

YASUMATSU, K., SAWADA, K., MORITAKA, S., MISAKI, M., TODA, J., WADA, J. & ISHII, K. (1972): Whipping and emulsifying properties of soybean products. *Agric. Biol. Chem., 36*, 719–729.

Acta Alimentaria, Vol. 18 (3), pp. 313—323 (1989)

REDUCTION OF PANEL VARIANCES BY A SIMPLE TWO-STEP NORMALIZATION PROCEDURE FOR GRAPHICAL LINE SCALE

J. WEISS[a] and H. ZENZ[b]

[a]Höhere Bundeslehr- und Versuchsanstalt für Wein- und Obstbau mit Institut für Bienenkunde, A-3400 Klosterneuburg. Austria
[b]Institut für Lebensmitteltechnologie, Universität für Bodenkultur, Peter-Jordan-Strasse 82, A-1190 Wien. Austria

(Received: 21 October 1988; accepted: 25 January 1989)

This paper presents the model of a two-step procedure to normalize sensory data gained from direct scaling. The aim of this procedure is to reduce panel variance. Data are normalized by means of a linear transformation in the form of $y = a + bx$. Normalization of the subject's different sensitivities is the first step; the second step comprises the normalization of the locations of each subject's sensitivity-normalized responses on the unstructured line scale. An example demonstrates the efficiency of the presented model: non-normalized data shows inhomogeneous standard deviations in the range of 6.13 to 15.88, corresponding to the scale range of 11.3 to 29.0%. The application of the presented two-step normalization procedure leads to a homogeneous standard deviation of 2.40 (4.4% of the scale range).

Keywords; two-step normalization procedure of sensory data, linear transformation

Commonly difference tests, ranking tests, descriptive tests or rating tests are employed to evaluate sensory quality of foodstuffs, whereby the problem to be investigated decides on the type of test. Of all these tests, rating tests are of main importance, using scales to document sensory judgements.

Scaling may be done either directly (by means of structured or unstructured scales) or indirectly (according to a special model or theory) (EKMAN & SJÖBERG, 1965; ANDERSON, 1976; WEISS, 1981a; BIRNBAUM, 1982; MCBRIDE, 1985; MCBRIDE, 1987a; MCBRIDE, 1987b). During the past years, studies on direct, unstructured scales were our main concern.

Unstructured scales represent a nonstructured continuum which, however, may be terminated by two ends. Sensory impressions are to be marked on this scale according to the individual sensitivity.

When using such a scale, not only problems by situational or contextural influences — which may lead to methodological bias — are inherent, but also the problem of how each single subject uses the scales has to be solved. This latter problem usually generates a wide variability in the responses of a sensory panel and may be coped with in applying following methods to reduce variability within a test panel:

— Normalization methods: All the individual structures allotted to the scale by the test panel are transformed to fit into a mutual scale structure.

— Selection and training of the panel: The aim is to implantate the above mentioned mutual scale structure into all subjects of the test panel by the help of suitable methods of selection and training.

— Non-parametric methods: Sensory responses of the test panel are ranked, the absolute values of the responses are disregarded.

Normalization methods have been investigated so far mostly with magnitude estimation (MOSKOWITZ, 1977; POWERS et al., 1981, WARREN, 1981; BUTLER et al., 1987). However, they also seem to be of general interest when unstructured scales are involved. This especially seems to be so when working with untrained test panels. Normalization methods should also be taken into consideration for structured scales and, therefore, for all direct scales.

This study presents a two-step normalization technique in a paradigmatical way. This two-step normalization technique undertakes to correct the responses of a test panel concerning the single subject's individual sensitivity (used range of scale) as well as the used zero-position on the scale of the used range of scale.

This procedure allows to reduce large and sometimes inhomogeneous variances within panel data and to present such data by means of simple statistical models. Further, the informative benefit of data analysis supported by graphical methods is demonstrated.

1. Materials and methods

1.1. Stimuli

Five sucrose solutions (20, 50, 60, 70 and 95 g dm^{-3} sucrose in tap water) were employed as "model system at five stimulus intensity levels" and were evaluated by each subject as to the intensity of the sensory attribute "sweetness".

1.2. Sensory evaluation method

1.2.1. Subjects. Subjects were five female and twenty male college students (age 18–23 years). They did not have any special training in sensory evaluations.

1.2.2. Scaling method. The scale was a 212 mm line scale arranged as an ascending diagonale from the lower left corner to the upper right corner of a 150×150 mm square. The lower and the upper end of the scale were denoted as "not at all sweet" and "extremely sweet", respectively. Subjects made a vertical mark across the scale to indicate their rating. After each sitting, the

ratings on the scale were transformed into values, using an overlay of the same length as the diagonal, but divided into 100 units, giving a response scale of 0–100 (WEISS et al., 1972; WEISS, 1981b).

1.2.3. Procedure. Subjects were given instructions in use of the scaling method. The asked question was "Estimate the intensity of the sensory attribute sweetness by using the unstructured line scale".

The subjects then took seat at one of two stations separated by partitions. Each station had five samples (sucrose solutions 20, 50, 60, 70 and 95 g dm^{-3}). Each sample was coded with a three digit random number. The subjects poured randomly the coded solutions into 150 cm^3 drinking glasses and tasted for sweetness. Back-tasting was allowed. There was also no time limit.

The whole procedure was repeated at the next day making four ratings for each sample and $(2 \times 2) \times 5 \times 25 = 500$ sensory responses in the entire set.

1.2.4. Design and analysis. The factors in this design were stimulus level (sucrose solutions), subjects, day of testing and replicate (within a day). This design was part of a larger project study in order to validate the performance of the unstructured line scale. In this paper we use as sensory responses for each sample the 25 mean sample responses (two replicates on two days) of each of the 25 subjects in the panel in order to show paradigmatically the performance of the proposed normalization method. The purpose of the normalization method is the normalization of the scale usage (used scale range and used position of the used scale range) by each subject and, therefore, the reduction of panel variance. We show the performance of the normalization method graphically (sensory responses of each subject for each of the five samples and box and whisker plots (CHAMBERS et al., 1983) of the sensory responses for each of the samples) and by One-way-ANOVA using a personal computer and standard software.

1.2.5. Normalization method. The normalization method is based on the hypothesis that the subjects in a sensory panel behave in scaling like instruments working with different sensitivities (range of the responses, used scale range) and different zero settings (position of the range of responses of each subject on the scale).

Therefore a two step normalization method, consisting of normalization of the individually used scale ranges and the locations of the used scale ranges, is necessary to normalize the individual responses of each subject. In the first step, the different sensitivities (used scale ranges) of the subjects are normalized. In the second step, the different locations of the sensivity-normalized responses of each subject on the scale are normalized. The treated problem is a calibration problem and the used normalization method is, in the form $y = a + bx$, a linear two-step transformation of scale range factors (b_i) and shift factors (a_i).

1.2.5.1. Sensitivity or scale range factors (b_i). – The sensitivity or scale range factors, normalize the individual sensitivities or scale ranges of each subject.

It is assumed that each subject works with a different but constant sensitivity during a session. According to this assumption the individual sensitivities of the subjects (E_i) are normalized to the averaged sensitivity of the panel (E_p).

Individual sensitivities (used scale ranges) of the subjects (E_i):

$$E_i = (R_{i,max} - R_{i,min})/c \tag{1}$$

$R_{i,max} - R_{i,min}$: used scale range, range of the responses (R_i) of the i-th subject ($i = 1, \ldots, n$)

n: number of subjects in the panel,

c: range of the intensities of the stimulus or samples (c is usually unknown and is eliminated in equation (3)).

Averaged sensitivity (used scale range) of the panel (E_p):

$$E_p = \Sigma E_i/cn \tag{2}$$

Individual sensitivity factors of the subjects (b_i):

$$b_i = E_p/E_i \tag{3}$$

Sensitivity normalized responses of the subjects ($R_{i,sens}$):

$$R_{i,sens} = b_i R_i \tag{4}$$

1.2.5.2. Shift factors (a_i) – The shift factors normalized the position of the range of the sensitivity normalized responses ($R_{i,sens}$) of each subject on the scale.

It is assumed that each subject work with a different but constant "zero setting" during a session. According to this assumption, the means of the sensitivity normalized responses of each subject are normalized to the panel mean.

Mean of the sensitivity normalized responses of each subject over all samples (M_i):

$$M_i = \Sigma R_{i,sens}/r \tag{5}$$

r: number of samples rated by each subject ($j = 1, 2, \ldots, r$)

Mean of the panel over all samples (M_p):

$$M_p = \Sigma M_i/n \tag{6}$$

Individual shift factors (a_i):

$$a_i = (M_i - M_p) \tag{7}$$

1.2.5.3 Two-step normalized sensory responses($R_{i,\,norm}$). According to equations 4 and 7 the two-step normalized sensory responses ($R_{i,\,norm}$) are:

$$R_{i,\,norm} = b_i R_i - a_i = R_{i,\,sens} - a_i \tag{8}$$

The two-step normalization method can easily be done on personal computers using following equations:

$$R_{i,\,sens} = \Sigma\,(R_{i,\,max} - R_{i,\,min})/(n(R_{i,\,max} - R_{i,\,min})) \tag{9}$$

$$R_{i,\,norm} = (\Sigma\,R_{i,\,sens} - (\Sigma\,\Sigma\,R_{i,\,sens})\,n)/r \tag{10}$$

In the given equations we first normalized according to the mean sensitivity (or mean used scale range) of the panel (E_p) and then according to the mean response (mean location of the used scale ranges) of the sensitivity normalized responses of the panel (M_p). Instead of E_p and M_p we could use also other values for E_p and M_p, for example the values of another panel, in order to compare the responses of this and the previous panel.

2. Results and discussion

The sensory responses (R_i) of the subjects for each tasted sucrose solution are represented in Fig. 1. From this figure we can clearly see, that each of the subjects could correctly differentiate between the intensities of the sweetness

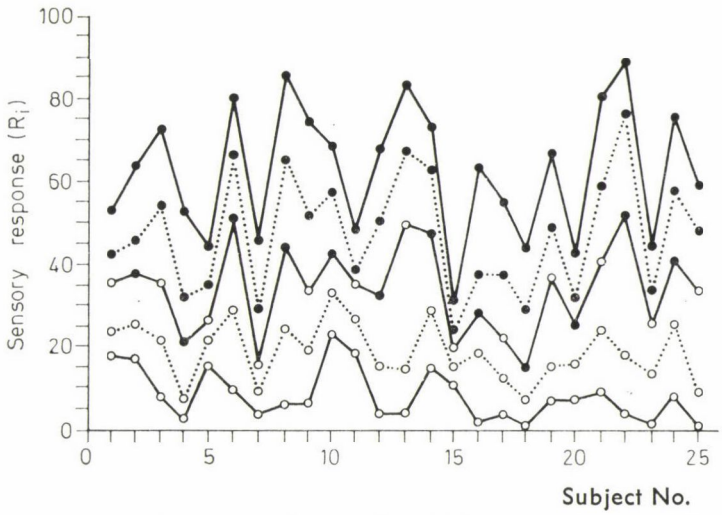


Fig. 1. Sensory responses (uncorrected data, R_i) of 25 subjects for five different sucrose concentrations. o—o—o: 20 g dm^{-3}, o--o--o: 50 g dm^{-3}, o--●--o: 60 g dm^{-3}, ●--●--●: 70 g dm^{-3} and ●—●—●: 95 g dm^{-3}

of the five sucrose solutions. But there are essential differences in the scale ranges used by each subject. For example, subject number 22 used a scale range of 85 (5–89) and subject number 15 used a scale range of 20 (11–31). The used mean scale range of the panel was 54.4. This is in accordance with published results (LAWLESS & MALONE 1986a and 1986b). But the used scale ranges varied considerably ($s^2 = 296$) and so did the sensory responses of the subjects. The variances varied from $s^2 = 37.6$ for 20 g dm^{-3} sucrose to $s^2 = 252.3$ for 95 g dm^{-3} sucrose (Table 1).

Table 1

Mean values and standard deviations of the panel responses for the five sucrose concentrations; uncorrected data (R_i), sensitivity normalized data ($R_{i,\,sens}$) and normalized data ($R_{i,\,norm}$)

Sucrose concentration (g dm^{-3})	R_i		$R_{i,\,sens}$		$R_{i,\,norm}$	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
20	8.43	6.13	10.63	10.56	10.63	1.89
50	19.13	7.12	21.39	11.65	21.39	2.39
60	34.10	10.89	35.87	11.76	35.87	2.99
70	47.44	14.35	49.03	10.83	49.03	2.64
95	62.80	15.88	65.00	10.56	65.00	1.89
Bartlett test	1.288		1.005		1.068	
Significance level	≤ 0.001		≤ 0.05		≤ 0.100	

The standard deviations, converted into percentage of scale range, are for these investigations up to 16% of the scale range.

These results are in accordance with those of LAWLESS and MALONE (1986b), giving an overall standard deviation of 16.7% for graphical line scales. The usage of different scale ranges can be interpreted as different sensitivities of the subjects in using the scale. Therefore we introduced the sensitivity normalization according to equations 1–3.

The results of the sensitivity normalization ($R_{i,\,sens}$) are represented in Fig. 2. From this figure we can see that the used scale ranges for all subjects are equal after sensitivity normalization of the responses. But there are positional differences between the used scale ranges of the subjects. For example subject number 11 used a scale range of 33–87 and subject number 25 used a scale range of 1–55. Therefore a locational shift factor was used in order to normalize the different individual locations of the sensitivity-normalized ranges used by the different subjects. This was the second normalization step.

In Fig. 3 the two-step normalized responses ($R_{i,\,norm}$) are represented. We can see from this figure that now the used scale ranges and also their locations are very similar, showing the efficiency of the two-step normalization

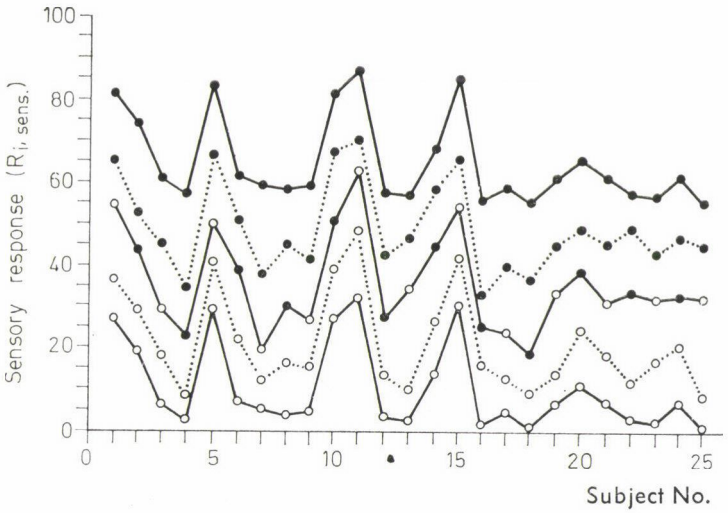


Fig. 2. Sensory responses (sensitivity normalized data, $R_{i,\ sens}$) of 25 subjects for five different sucrose concentrations. ○—○—○: 20 g dm^{-3}, ○--○--○: 50 g dm^{-3}, ○-●--○: 60 g dm^{-3}, ●--●--●; 70 g dm^{-3} and ●—●—●: 95 g dm^{-3}

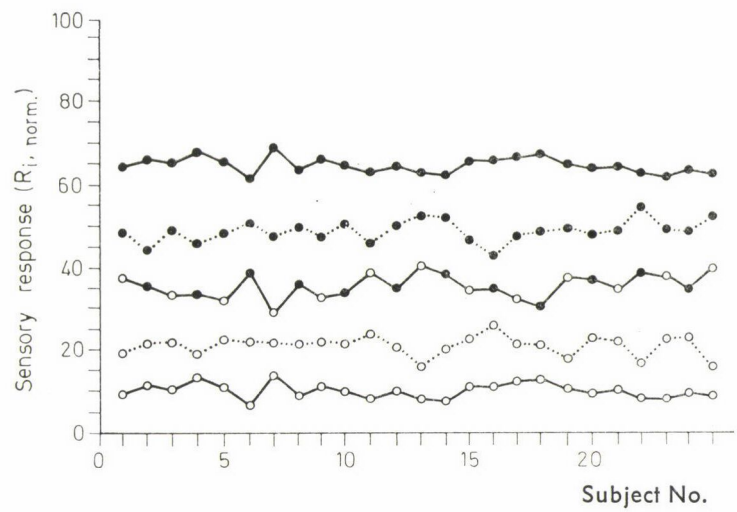


Fig. 3. Sensory responses (normalized data, $R_{i,\ norm}$) of 25 subjects for five different sucrose concentrations. ○—○—○: 20 g dm^{-3}, ○--○--○: 50 g dm^{-3}, ○-●--○: 60 g dm^{-3}, ●--●--●: 70 g dm^{-3} and ●—●—●: 95 g dm^{-3}

method, resulting in an essential reduction of the variance of the normalized sensory responses ($R_{i,\ norm}$) (Table 1). Formal statistical One-Way-ANOVAs show the mean squares (MQ) "between samples (sucrose solutions)" and "within samples". The MQ "within samples" is a pooled variance and characterizes the variance of the sensory responses of the panel (panel variance).

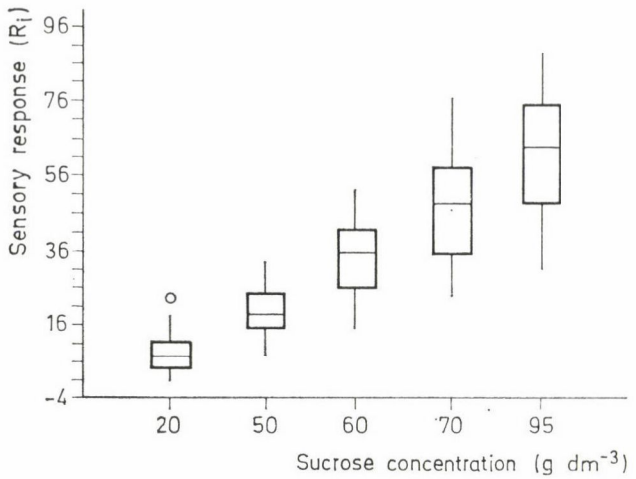


Fig. 4. Box and whisker plots of uncorrected data (R_i) of all subjects for five different sucrose concentrations

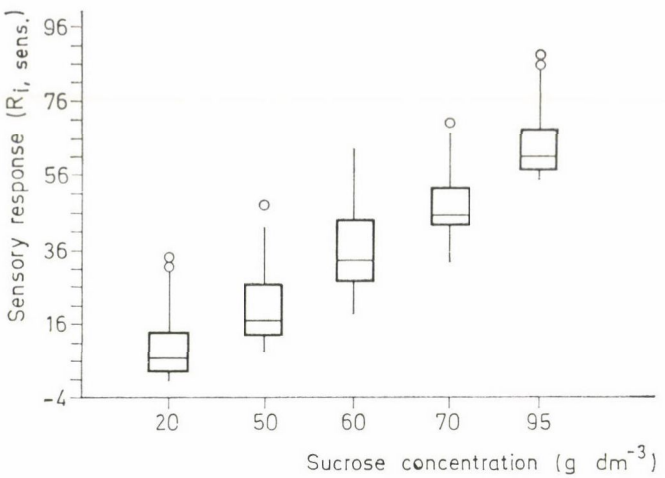


Fig. 5. Box and whisker plots of sensitivity normalized data ($R_{i,\,sens}$) of all subjects for five different sucrose concentrations

For the not normalized sensory responses (R_i, Table 1) the variances "within samples" are highly inhomogeneous as is seen from Fig. 4. Therefore the pooled variance (MQ within samples) is not a correct measure of the panel variance, varying from $s^2 = 37.6$ for 20 g dm^{-3} sucrose solution to $s^2 = 252.3$ for 95 g dm^{-3} sucrose solution.

Sensitivity normalization ($R_{i,\,sens}$, Table 1 and Fig. 5) comes up with homogeneous variances "within samples" over all sucrose solutions. The mean square being 122.88 according to a mean standard deviation of $s = 11.09$ or 20.39% of used scale range.

Table 2

One-way-analyses of variances of the uncorrected (R_i), sensitivity normalized ($R_{i,\,sens}$) and normalized data ($R_{i,\,norm}$)

Source	Sum of squares	DF	Mean square	F-ratio
Uncorrected data (R_i)				
Between samples	47 107·585	4	11 776·896	88.55
Within samples	15 959·615	120	132·997	
Sensitivity normalized data ($R_{i,\,sens}$)				
Between samples	46 679·852	4	11 669·963	94.97
Within samples	14 746·000	120	122·883	
Normalized data ($R_{i,\,norm}$)				
Between samples	46 679·852	4	11 669·963	2 028 50
Within samples	690·355	120	5·753	

While sensitivity (scale range) factors made the variances "within samples" homogeneous, the shift factors reduced the variances "within samples" dramatically; compare MQ = 122.88 for the $R_{i,\,sens}$-values with MQ = 5.8 for the $R_{i,\,norm}$-values in Table 2. The standard deviation of the two-step normalized sensory responses ($R_{i,\,norm}$) was $s = 2.40$ or 4.44% of the used scale range in comparison with the not normalized sensory responses (R_i) ranging from $s = 6.13$ to 15.88 or 11.27 to 29.19% of the used scale range.

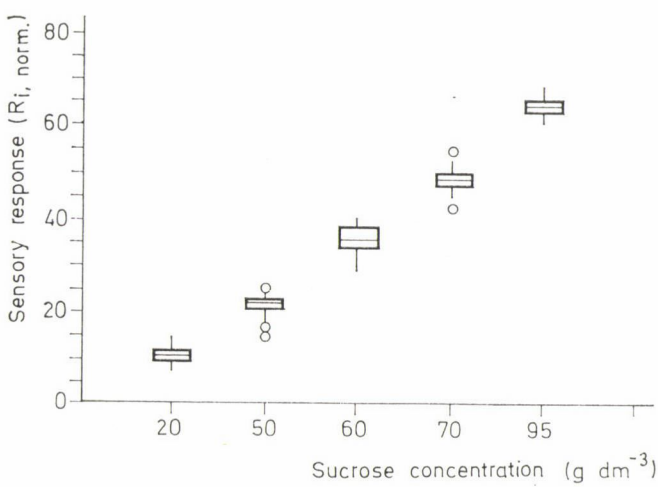


Fig. 6. Box and whisker plots of normalized data ($R_{i,norm}$) of all subjects for five different sucrose concentrations

The box and whisker plots of the sensory data (Figs. 4–6) clearly demonstrate the performance of the two-step normalization method visually. This figures are autointerpretative and in this sense they are more informative than formal One-Way-ANOVAs and multiple comparisons (not shown here). As can be seen by this figures, only the "sample ranges" of the two-step normalized sensory responses ($R_{i,\,norm}$, Fig. 6) do not overlap, indicating the discriminating power of the subjects (measuring instrument) by using the unstructured line scale. This information is equivalent with Fig. 1 in showing that the subjects could clearly differentiate between the tasted sucrose solutions. An information which is also obtainable with nonparametric tests.

3. Conclusions

The advantage in using the proposed two-step normalization method lies in the fact that it produces quantitative data of small variances which usually are homogeneous. This allows to apply advanced statistical methods which postulate normality of data and homogeneity of variances (SNEDECOR & COCHRAN, 1971).

A further advantage of the presented method is that it may be visually verified by means of a graph (sensory responses vs. subject No., Figs. 1–3). The anticipated results of the proposed method may be estimated in advance. If, however, the subjects' responses are inconsistent — which may be easily taken from the graph — the proposed method will of course not allow to correct this inconsistency.

It is assumed that the given stimuli show neglectable variability. If, however, such variability is large, then special procedures have to be applied. A more universe model, based on strictly statistical considerations and also taking into account the problem of different test panels, is presented by MILLER (1987).

When scaling directly it cannot be discerned whether the ranges of the scale (scale utilization) which were used by a given subject and their position on the scale are the result of the given subject's process of perception (psychophysical function) or of his way to make visible on the scale the sensitivities he perceived (judgement function) (BIRNBAUM, 1982).

Sensory data gained form direct scaling usually is to be regarded as relative data, relevant only to the present contextural effects (RISKEY, 1986). This relativity is not affected by the suggested transformation. The quotients of differences of the nonnormalized and normalized values are identical. It is only the large variability that is reduced to make contextural effects come forward more clearly.

Literature

ANDERSON, N. H. (1976): Integration theory, functional measurement and the psychophysical law. — in: GEISSLER, H. G. & ZABRODIN, Y. M.: *Advances in psychophysics*. VEB Deutscher Verlag der Wissenschaften, Berlin, pp. 93–130.

BIRNBAUM, M. H. (1982): Problems with so-called "Direct" scaling. — in: KUZNICKI, J. T., RUTKIEWIC, E. F. & JOHNSON, R. A. (1982): *Selected sensory methods: problems and approaches to measuring hedonics. Special Technical Publication 773*. ASTM, Philadelphia, pp. 34–48.

BUTLER, G., POSTE, L. M., WOLYNETZ, M. S., AGAR, V. E. & LARMOND, E. (1987): Alternative analyses of magnitude estimation data. *J. Sensory Studies, 2*, 243–257.

CHAMBERS, J. M., CLEVELAND, W. S., KLEINER, B. & TUKEY, P. A. (1983): *Graphical methods for data analysis*. Wadsworth & Brooks Cole Publishing Company; Pacific Grove.

EKMAN, G. & SJÖBERG, L. (1965). Scaling. *Ann. Rev. Psychol., 16*, 451–474.

LAWLESS, H. T. & MALONE, G. J. (1986a): The discriminative efficiency of common scaling methods. *J. Sensory Studies, 1*, 85–98.

LAWLESS, H. T. & MALONE, G. J. (1986b): A comparison of rating scales: sensitivity, replicates and relative measurement. *J. Sensory Studies, 1*, 155–174.

MCBRIDE, R. L. (1985): Sensory measurement: an introductory overview. *CSIRO Fd Res. Quart., 45*, 59–63.

MCBRIDE, R. L. (1987a): Psychophysics as measurement science, sensory science, food science. *Chemistry and Industry, 1*, 25–30.

MCBRIDE, R. L. (1987b): Taste psychophysics and the Beidler equation. *Chemical Senses, 12*, 323–332.

MILLER, A. J. (1987): Adjusting taste scores for variations in use of scales. *J. Sensory Studies, 2*, 231–242.

MOSKOWITZ, H. R. (1977): Magnitude estimation: notes on what, how, when and why to use it. *J. Fd Quality, 3*, 195–227.

POWERS, J. J., WARREN, C. B. & MASURAT, T. (1981): Collaborative trials involving three methods of normalizing magnitude estimations. *Fd Sci. Technol., 14*, (2), 86–93.

RISKEY, D. R. (1986): Use and abuse of category scales in sensory measurement. *J. Sensory Studies, 1*, 217–236.

SNEDECOR, G. W. & COCHRAN, W. G. (1971): *Statistical methods*. Iowa State University Press, Ames (Iowa, USA), pp. 229–338.

WARREN, C. B. (1981): Development of fragrances with functional properties by quantitative measurement of sensory and physical parameters. — in: MOSKOWITZ, H. R. & WARREN, C. B. *Odor quality and chemical structure. ACS Symposium Series 148*, American Chemical Society, Washington DC. pp. 57–77.

WEISS, J., WILLISCH, E., KNORR, D. & SCHALLER, A. (1972): Ergebnisse von Untersuchungen bezüglich der differenzierenden Wirkung einer sensorischen bewertenden Prüfmethode gegenüber einer sensorischen Rangordnungs-Prüfmethode am Beispiel von Apfelsaft und Birnennektar. *Confructa, 17*, 237–250.

WEISS, J. (1981a): Rating scales in the sensory analysis of foodstuffs. — Part I. Methodological considerations. *Acta Alimentaria, 10*, 389–394.

WEISS, J. (1981b): Rating scales in the sensory analysis of foodstuffs. — Part II. Application of a rating method with unstructured scale. *Acta Alimentaria, 10*, 395–405.

Acta Alimentaria, Vol. 18 (3), pp. 325—330 (1989)

DESIGNED PROTEIN MODIFICATION BY ENZYMATIC TECHNIQUE

GY. HAJÓS[a, c], A. HALÁSZ[a] and F. BÉKÉS[b]

[a]Department of Biochemistry, Central Food Research Institute, H-1025 Budapest, Herman Ottó út 15. Hungary
[b]Department of Biochemistry and Food Technology, Technical University of Budapest. H-111 Budapest, Műegyetem rkp. 3. Hungary

(Received: 22 November 1988, accepted: 25 January 1989)

Casein hydrolysate was enriched in methionine by enzymatic peptide modification (EPM) and the structural changes in the products were investigated by isoelectric focusing. For the evaluation of the electrophoretograms, a computer assisted method has been elaborated. The results corroborated that transpeptidation took place during the EPM treatment.

Keywords: enzymatic peptide modification, EPM, Met-enrichment, computer evaluated isoelectric focusing

The ability of proteinases to catalyse peptide bond synthesis has a historical background (SAWJALOW, 1907; WASTENEYS & BORSOOK, 1930) and has also awoke a great interest recently (FUJIMAKI et al., 1977; SUKAN & ANDREWS, 1982; LUDWIG et al., 1981; ASO et al., 1985). The enzymatic peptide modification proved to be a suitable enzymatic process to obtain protein product of designed amino acid composition. The reaction mechanism of this enzymatic process and the chemical nature of incorporation during EPM is, however, still not yet clarified. The main force of this enzymatic reaction is believed to be by some authors condensation (WIELAND et al., 1960; YAMASHITA et al., 1973), whereas by other transpeptidation (HOROWITZ & HAUROWITZ, 1959; GOLOLOBOV et al., 1981). Moreover noncovalent forces have also been reported to play an important role in these enzyme-catalysed reactions (HOFSTEN & LALASIDIS, 1976; NOAR & SHIPE, 1984).

Our recent results revealed the covalent nature of incorporation of the amino acids (HAJÓS et al., 1988a) and transpeptidation in the course of EPM was also verified (HAJÓS & HALÁSZ, 1982; DELINCÉE & HAJÓS, 1984; HAJÓS, 1986; HAJÓS et al., 1988b).

In this study, changes in the protein structure during EPM were followed by isoelectric focusing. The electrophoretograms representing the separation

[c]To whom correspondence should be addressed

of the peptide zones by their net charges have been evaluated by using computer analysis.

1. Materials and methods

1.1. Casein

Casein was purchased from Reanal (Hungary). α-Chymotrypsin and L-Methionine ethyl ester from Sigma were used.

1.2. Preparation of casein hydrolysate

Casein was hydrolysed with α-chymotrypsin at pH 7.5 and 37 °C for 4 h with stirring.

1.3. Enzymatic peptide modification

Casein hydrolysate (5 g) was mixed at pH 6.0 with α-chymotrypsin (50 mg) without (EPM 1) or with addition (EPM 2) of L-Methionine ethyl ester (1 g). The reaction mixture was filled up to 20 cm^3 and was incubated without shaking at 37 °C for 16 h. The whole mixture after incubation was dialysed through a cellophane membrane against distilled water for 2 days. The nondialysable fraction was liophylised at 5–7 °C.

1.4. Determination of amino acid composition

An aliquot of the sample was hydrolysed with 6M hydrochloric acid in an evacuated tube at 105 °C for 24 h.

The hydrochloric acid-hydrolysed samples were analysed for amino acids with a Biotronik LC 2000 amino acid analyser. (Resin: BTC 3118, 11 μm).

1.5. Thin layer isoelectric focusing

Isoelectric focusing on Sephadex G-75 Superfine was carried out with 6M urea added to the gel (HAJÓS & DELINCÉE, 1983). The samples for focusing were dissolved in 8M urea. The prints obtained from the separations on Sephadex were stained for protein with Coomassie Brillant Blue G–250 (RADOLA, 1973).

1.6. Computer program

Numerical comparison of distribution of protein fractions — i.e. the "homology" of samples — was done by a computer program. The term "homology" (H%) describes the differences in mobilities and intensities of each electrophoretic band of the two samples compared:

$$H\% = \frac{2\left(\sum_{1=1}^{k} D_i\right)}{N_1 + N_2} 100$$

where k: number of pairs of bands with a difference in mobility of smaller than ε. (For ε 0.5% relative mobility was taken);

D_i: weighting factor which depends on the difference of intensities of the two homologue bands;

N_1 and N_2: number of bands in the two electrophoretograms compared.

Computer program was written in BASIC for IBM PC.

Beside the above mentioned calculation a graphical evaluation was also carried out. Thus, the image of electrophoretic separation was depicted using the digitalised relative mobility and intensity data.

2. Results

Casein hydrolysate (α-chymotryptic) was incubated in the presence of α-chymotrypsin without (EPM 1) and with (EPM 2) addition of L-methionine ethyl ester under appropriate reaction conditions.

Methionine-content of casein, casein-hydrolysate and products of the enzymatic peptide modification are summarized in Table 1. As this comparison shows Met content in the EPM-product with methionine incorporation (EPM 2) was found to be more than twice as high as that of the substrate protein.

Table 1

Methionine content of the samples

Sample	Methionine[a] (%)
Casein	2.3
Casein-hydrolysate	2.2
EPM 1	2.2
EPM 2	4.8

[a] The methionine content of the samples is given as per cent of the total amino acid content

Changes of charge properties of peptides during EPM processes were detected by isoelectric focusing in the presence of urea and the electrophoretic patterns were evaluated by a computer-assisted method.

The computer graphs of casein, casein-hydrolysate and the two EPM-products (EPM 1 and EPM 2) are shown in Fig. 1.

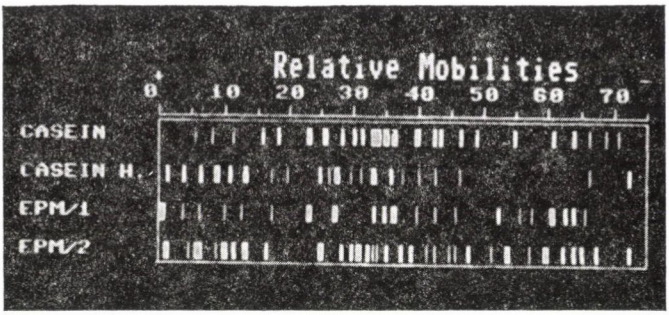


Fig. 1. Computer graph of isoelectric focusing

Homology matrix of the four samples compared is shown in Table 2. Graphical interpretation of the separations together with the homologous and nonhomologous bands are presented for the comparison of casein hydrolysate with EPM 1 (Fig. 2) and for the comparison of the same hydrolysate with EPM 2 (Fig. 3), respectively. Comparison of the two products with the casein hydrolysate gave a homology value of 20.0 for EPM 1 and 28.8 for EPM 2, respectively.

Table 2

Homology matrix of the four samples compared

	Casein	Casein hydrolysate	EPM 1	EPM 2
Casein	100.0	19.2	24.0	29.7
Casein-hydrolysate	19.2	100.0	20.0	28.8
EPM 1	24.0	20.0	100.0	21.6
EMP 2	29.7	28.8	21.6	100.0

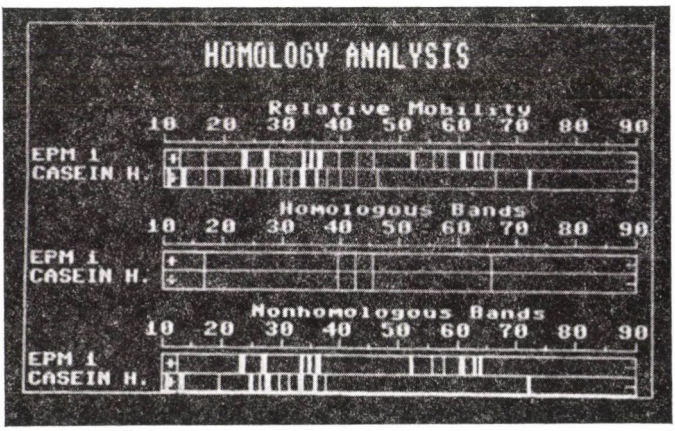


Fig. 2. Homologous and nonhomologous bands of casein hydrolysate and EPM 1

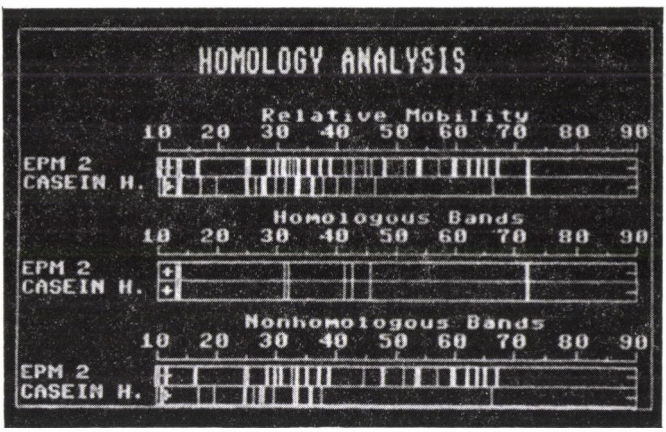


Fig. 3. Homologous and nonhomologous bands of casein hydrolysate and EPM 2

3. Discussion

The separation by net charge of protein fractions of both EPM products was found to be significantly different from that of the substrate. Since the average molecular mass remained practically unchanged during the EPM process (DELINCÉE & HAJÓS, 1984), these differences (see Fig. 1) indicate that transpeptidation does take place in the reaction.

On the basis of the large number of nonhomologous bands in both cases (Fig. 2. and Fig. 3) we conclude that a great number of peptide bonds was cleaved and formed due to a considerable transpeptidation. It is interesting to note that, in this respect, the samples both with and without methionine addition behaved in an analogous way. This suggests that transpeptidation takes place in all EPM reactions.

The methionine content of EPM 2 is greater by only 2.6% than that of EPM 1, nevertheless the peptide fractions of the EPM-product without amino acid incorporation (EPM 1) and the Met-enriched product (EPM 2) differed from each other remarkably (see Table 2). This is why we suppose, that the presence of methionine in the reaction mixture and its incorporation give rise to a new type of transpeptidation, namely modify the sequential position of the cleaved and formed peptide bonds.

*

We thank Ms IDA ÉLIÁS for excellent experimental performance, and thanks are due to Ms ÁGNES BARÁTH for the amino acid analyses.

Literature

ASO, H., KIMURA, H., WATANABE, M. & ARAI, S. (1985): Chemical properties of enzymatically modified proteins produced from soy protein by covalent attachment of methionine. *Agric. biol. Chem.*, *49*, 1649–1654.

DELINCÉE, H. & HAJÓS, GY. (1984): Investigation of plasteins by SDS polyacrylamide gel electrophoresis. *Acta Alimentaria*, *13*, 309–313.

FUJIMAKI, M., ARAI, S. & YAMASHITA, M. (1977): Enzymatic protein degradation and resynthesis for protein improvement. *Adv. Chem. Ser.*, *160*, 156–184.

GOLOLOBOV, M. Y., BELIKOV, V. M., VITT, S. V., PASKONOVA, E. A. & TITOVA, E. F. (1981): Transpeptidation in concentrated solution of peptic hydrolysate of ovalbumin. *Nahrung*, *25*, 961–967.

HAJÓS, GY. (1986): Incorporation of essential amino acids into protein by enzymatic peptide modification (EPM). *Nahrung*, *30*, 418–419.

HAJÓS, GY. & HALÁSZ, A. (1982): Incorporation of L-methionine into casein hydrolysate by enzymatic treatment. *Acta Alimentaria*, *11*, 189–197.

HAJÓS, GY. and DELINCÉE, H. (1983): Structural investigation of radiation induced aggregates of ribonuclease. *Int. J. Radiat. Biol.*, *44*, 333–342.

HAJÓS GY., ÉLIÁS, I. & HALÁSZ, A. (1988a): Methionine-enrichment of milk protein by enzymatic peptide modification. *J. Fd Sci.*, *53*, 739–742.

HAJÓS, GY., MIETSCH, F. & HALÁSZ, A. (1988b): Change of degree of hydrolysis in the course of enzymatic peptide modification. *Acta Alimentaria*, *17*, 265–270.

HOFSTEN, B. V. & LALASIDIS, G (1976): Protease catalyzed formation of plastein products and some of their properties. *J. agric. Fd Chem.*, *24*, 460–465.

HOROWITZ, J. & HAUROWITZ, F. (1959): Mechanism of plastein formation. *Biochem. biophys. Acta*, *33*, 231–238.

LUDWIG, E., KRAUSE, W., NÖTZOLD, H. & TEBLING, F. (1981): Enzymatische Modifizierung (Plasteinsynthese) von Ackerbohnen-protein. *Nahrung*, *25*, K15–K17.

NOAR, S. R & SHIPE, W. F. (1984): A study of the forces involved in the incorporation of L-methionine into soy protein by the one-step plastein-like process. *J. Fd Sci.*, *49*, 1316–1318.

RADOLA, B. J. (1973): Isolectric focusing in layers of granulated gels. *Biochim. biophys. Acta*, *295*, 412–428.

SAWJALOW, W. W. (1907): Über das Plastein. *Z. physiol. Chem.*, *54*, 119–150.

SUKAN, G. & ANDREWS, A. T. (1982): Application of the plastein reaction to caseins and to skim-milk powder. *J. Dairy Res.*, *49*, 279–293.

WASTENEYS, H. & BORSOOK, H. (1930): The enzymatic synthesis of protein. *Physiol. Rev.*, *10*, 110.

WIELAND, T., DETERMANN, H. & ALBRECHT, E. (1960): Untersuchungen über die Plastein-Reaktion, I. Isolierung einheitlicher Plasteinbausteine. *Justus Liebigs Annln Chem.*, *633*, 185.

YAMASHITA, M., ARAI, S., TANIMOTO, S. & FUJIMAKI, M. (1973): Condensation reaction occurring during plastein formation by α-chymotrypsin. *Agric. biol. Chem.*, *37*, 953–954.

BOOK REVIEWS

Biological role of plant lipids

P. A. Biacs, K. Gruiz, & T. Kremmer (Eds)

Akadémiai Kiadó, Budapest and Plenum Publishing Corporation,
New York and London, 1989, 625 pages

The Eighth International Symposium on the Biological role of plant lipids took place at Budapest, Hungary, July 25–28, 1988.

The scientific program, divided into 14 sessions, included 11 general lectures, 75 oral lectures and 70 posters. Due to the large number of papers the Symposium was held in two parallel sections.

Lectures were given on traditional areas of plant lipid research, as lipid metabolism, structure and function of lipids. Special attention was paid to the session dealing with biocides and their interaction with plant lipids and with the effect of environmental stress of plant lipids. Highlights of the Symposium were the new discoveries of molecular biology and the genetics of plant lipids. A special session was devoted to the growing importance of oleo-biotechnology.

The Proceeding contains the lectures in 7 chapters. The first chapter deals with the lipid metabolism in 42 lectures, the second with the structural and functional organization of lipids in 23 lectures; the third with the biosynthesis and function of prenyllipids; the fourth with the carrier proteins, genetics of plant lipids in 10 lectures; the fifth with the biocides, interaction with plant lipids in 13 lectures; the sixth with the biotechnology of lipids, nutritional aspects in 11 lectures; the seventh with the development, environment, stress in 28 lectures. The Proceeding also contains the author index, subject index and the index of taxa.

The book may be utilized not only in the work of researchers but also in higher education.

I. Varsányi

Colour and optical properties of foods

M. Kent (Ed.)

Food Science Publishers, England, 1989; 118 pages

A bibliography compiled as part of the COST90bis Project on the colour and optical properties of foods covering the period from the earliest known reference up to 1987. This project was COST90bis, the second stage of a European cooperation in the field of physical properties of foods. (COST is an acrinym for "European Cooperation in Scientific and Technological Research", subjects concerned with Food being designated by the first digit "9"; physical properties, being the first project in this field, is designated "0".

Bis refers to the second stage of the Project.) Other properties studied in COST90bis were electrical, mechanical and diffusional properties. In the main part of the bibliography the references in English, translated from the original where necessary, are arranged in alphabetical order of authors' names and numbered sequentially. For additional information, the original (18) languages of the non-English texts are indicated by corresponding letters in the right hand margin. In the second section the references are classified according to type of foodstuffs.

This bibliography contains more than 1000 referenda. Most of the references (217) refer to meat products; the next largest group is the meat with 177 references. The less reference (1) was given to bread, beans and to apricot juice.

This collection of published papers is very useful both for the industry and research but the high school education may also utilize it well.

É. SZÁNTÓ-NÉMETH

ANNOUNCEMENT

INTERNATIONAL CONGRESS OF REFRIGERATION

The XVIIIth Congress of the International Institute of Refrigeration will be held at the Montreal Convention Centre from August 10 to 17, 1991, on the theme of "NEW CHALLENGES IN REFRIGERATION".

The Congress, to be held there at the invitation of Agriculture Canada, will be the first such International Institute of Refrigeration (IIR) event in Canada since the organization's founding in 1908. As the Canadian delegate to the IIR, the Director of the Saint-Hyacinthe Food Research and Development Centre, Claude Aubé, will act as the Chairman.

Under the International Convention, the IIR holds an international congress every four years, at the invitation of a member country. The 1987 Congress was held in Vienna, the 1983 Congress in Paris, and the previous one in Venice, in 1979.

The Canadian organizing committee is innovating this year by adding a new series of presentations on the many applications of food irradiation combined with refrigeration techniques.

Over 1200 delegates from 50 to 60 countries will take part in the Congress in Montreal, attending nearly 500 technical and scientific presentations. In addition, exhibitors will be displaying new products and advanced technolgies.

For further information, please contact:
Steve Bittner, Co-ordinator
XVIIIth International Congress of Refrigeration Inc.
St-Hyacinthe, Quebec, Canada
J2S8E3
Telephone: (514) 773–1105
Fax: (514) 773–8461

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

RECENTLY ACCEPTED PAPERS

Results of measurements of deformation versus load relations in tin cans
KÖRMENDY, I. & FERENCZY, I.

Preparation and functional properties of acetylated rapeseed proteins
GWIAZDA, S., DABROWSKI, K. J., KLEPACKA, M. & RUTKOWSKI, A.

S-metabolism of methionine-rich yeasts
HALÁSZ, A., MÁTRAI, B. & MUAYAD, A.

Results of a sensory collaborative test of some food products
MOLNÁR, P.

NOTICE TO CONTRIBUTORS

General. Manuscripts in English or Hungarian should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

Title. The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

Summary. The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

Text. The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

Symbols. Units, symbols and abbreviations should be used according to SI (System International) rules. Unusual abbreviations must be explained in the text or in an appendix.

Figures and tables. Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

References. The reference list (Literature) should be in alphabetical order as follows:

Periodicals: Names and initials of all the authors; year of publication in parentheses; colon; title of the paper; title of the periodical; inclusive page numbers.

Books: Names and initials of all the authors; the year of publication in parentheses; colon; title of the book; publishing firm, place of publication; inclusive page numbers.

Detailed instructions for publishing in Acta Alimentaria are available from the Editorial Office.

Authors will receive one set of proofs which must be corrected and returned at the earliest convenience to the Editorial Office. In this phase major alterations of the text cannot be accepted.

Offprints. We supply 100 offprints free of charge. Additional copies can be ordered.

No page charges are levied on authors or their institutions.

Acta Alimentaria is surveyed by Current Contents/Agriculture, Biology and Environmental Sciences, ASCA, BIOSIS, Nutrition Abstracts and Reviews.

ACTA ALIMENTARIA

VOLUME 18 No. 3 — 1989

CONTENTS

Index: 26.039

ACTA

ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 18 **December 1989** **Number 4**

Akadémiai Kiadó
Budapest

ISSN 0139—3006

CODEN ACALDI

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

Distributor:

KULTURA, Hungarian Foreign Trading Company
P.O. Box 149, H-1389 Budapest 62, Hungary

Publication programme, 1990: Volume 19 (4 issues).
Subscription prices per volume: US $ 130,00 plus 15% postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 18

1989

AKADÉMIAI KIADÓ
BUDAPEST

CONTENS

Vol. 18.
1989

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 18

1989

AKADÉMIAI KIADÓ
BUDAPEST

Acta Alimentaria, Vol. 18 (4), pp. 333—351 (1989)

RESULTS OF MEASUREMENTS OF DEFORMATION VERSUS LOAD RELATIONS IN TIN CANS

I. KÖRMENDY[a] and I. FERENCZY[b]

[a]Department of Canning Technology, University of Horticulture and Food Industry H-1118 Budapest, Ménesi út 45. Hungary
[b]Canning Factory, H-7030 Paks, Tolnai u. 2. Hungary

(Received: 1 February 1988; accepted: 30 December 1988)

The authors measured the deformation (change of volume, ΔV) in cans, type ∅ 99–1/1 with jackets and end plates of different thicknesses (0.18; 0.2, 0.22, 0.24 mm) as a function of load (pressure difference, Δp). Load cycles in conformity with industrial practice were applied in the measurements, taking into account the critical loads causing marked permanent deformation. By executing parallel measurements the variances of volume change were also obtained. Variances were generally non-homogeneous. The average dispersion obtained from all measurements was 2.44 cm^3 being about 4–5% of the maximal change of volume. Results of the measurements are presented by curves or sets of curves representing relations $\Delta V = f(\Delta p)$. Results can be used to determine by calculation the pressure differences and deformations developing during heat treatment, to consider in advance the effects of reduced plate thickness, by incorporating the results in known calculational methods.

Keywords; deformation of cans, sterilization of food, heat treatment

In the present study the aim of the authors was the complete measurement of the relations between the load on cylindrical cans and their deformation with regard to establishing proper calculation methods.

The instrument and the principle of measurement developed by the authors is essentially based on the original paper of HEISS (1955) and its later interpretation by ROGACSEV and BABARIN (1983). First adoption in Hungary was made by KÖRMENDY (1982), followed by the results of DELI (1982), who investigated cans of type 5/1 and 7/2 (volume abt. 4.3 and 3.2 dm^3, respectively).

Further investigations have been carried out by FERENCZY and KÖRMENDY (1984) and the results were included in the present paper for evaluating the experiments and for mathematical statistical analyses. Recent experiments constitute further development of earlier investigations. A substantial part of the results was incorporated in the doctoral thesis of FERENCZY (1986).

According to the knowledge of the authors it is principally new here that a complete systematic series of load cycles for a given can was set up and the deformation of cans was measured in this way. In altering the load the industrial heat treatment practice was taken into account.

1. Materials and methods

1.1. Types of the experiments and parameters of cans used in the experiments

Four types of experiments can be distinguished, these are listed in Table 1. The same contains the (nominal) parameters of the cans used in the experiments and the number of parallel measurements. The characteristics of the tin plates used to manufacture the cans are listed in Table 2. In Table 1 the data belonging to results hitherto published are marked (+) (FERENCZY & KÖRMENDY, 1984).

Table 1

Serial number of experiments, data pertinent to type ∅ 99–1/1 cans, type of experiment, number of parallel measurements

Serial number	Nominal thickness of plate (mm)		Type of measurement, number of parallels		
	cylinder jacket	end plate	A B	C	D
1 +	0.24	0.24	A, 3	3	3
2 +	0.22	0.24	A, 2	3	3
3 +	0.22	0.22	A, 2	3	3
4 +	0.20	0.20	A, 3	3	3
5 +	0.18	0.18	A, 2	3	3
6	0.20	0.24	B, 3	—	—
7	0.20	0.22	B, 3	—	—
8	0.20	0.20	B, 3	—	—

Further information is given in para. 1.1. in relation to the type of the can

Meaning of symbols A, B, C, D is explained under paras 1.3., 1.4. and 1.5.

Table 2

Data of the tinned steel plates used for can manufacture

Nominal size (mm)	Tin content (g m^{-2})	Mark of coating mass category[a]	Actual thickness (mm)	Temper (hardness) (in degrees Rockwell)	Category of temper (hardness)[a]
0.24×650×790	10.81	E 5.6/5.6	0.239	59.5	T 57
0.22×727×844	5.66	E 2.8/2.8	0.215	63.0	T 61
0.20×712×880	8.50	D 5.6/2.8	0.204	60.0	T 57
0.18×708×765	11.13	E 5.6/5.6	0.180	64.0	T 61

[a] According to Euronorm 145–78

In the experiments the pressure differences were varied and the difference is marked Δp.

$$\Delta p = p - p_K$$

where p: internal pressure in the can, bar

p_K: pressure of the medium surrounding the can, called external pressure, in this case atmospheric pressure, bar

Positive values of Δp are referred to as overpressure. When the can is under vacuum, Δp has a negative value.

Differences in volume belonging to the pressure differences are marked ΔV, $(\Delta V = V - V_0)$,

where V: the volume of the can at a given pressure difference, cm^3

V_0: initial volume of the can at zero difference in pressure, cm^3

In these experiments the value of V_0 was 860 cm^3.

The cans used in the experiments were of type ⌀ 99–1/1, made of tinned steel plates. This designation indicates a can of 99 mm nominal diameter and 118.5 m height. These data and the cylindrical jacket and end plates of the cans comply to the specifications of the relevant HUNGARIAN STANDARD (1980). They were closed empty in the Canning Factory, Paks, Hungary, on the automated machine line Type Blema (GDR).

The steel plates were checked in conformity to Euronorm 145–78 standard.

No cans for the experiments had been previously used and they were not exposed to the traditional industrial pressure test. All cans have been used only once in an experimental (multiple) loading cycle, thus a parallel measurement is understood as repetition on a new can.

1.2. Description of the experimental equipment

Figure 1 presents the technical scheme of the instrument.

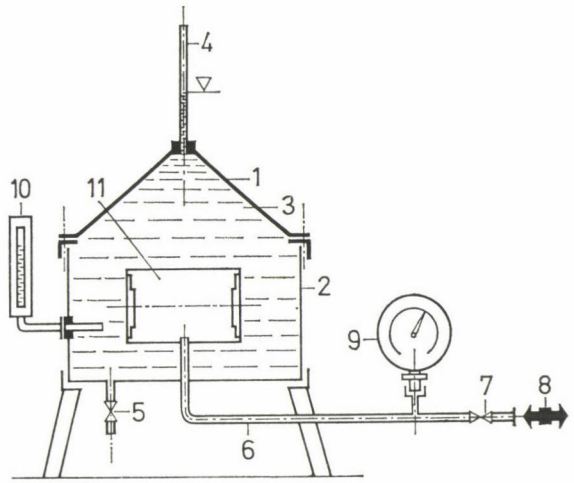


Fig. 1. Schematic diagram of the measuring instrument. 1: cover; 2: wall of the measuring chamber; 3: chamber of the instrument; 4: glass tube; 5: discharge valve 6: air conduit; 7: air valve; 8: air inlet or outlet; 9: manovacuum gauge; 10: thermometer; 11: can

Using the same marking as in Fig. 1 the instrument operates as follows:

The empty can is placed in the container of the instrument, marked **3**. In the wall of the can a copper tube of small diameter is soldered, marked 6. The internal space of the can is connected to a compressor or vacuum pump through a threaded joint, tube and valves. The difference between the atmospheric pressure and the pressure prevailing in the can is measured with a manovacuum meter, marked 9. The range of the overpressure is: 0–1.47 bar, of the vacuum: 0–0.98 bar.

Grade of accuracy: 1, scale division: 0.049 bar. The boundary surfaces of the can are not hindered in their free displacement in the equipment.

The rigid chamber of the equipment (3) is filled up with water while the air is carefully exhausted. A glass tube (meniscus tube), marked 4, with scale divisions of 0.1 cm^3, is connected to the body. The glass tube serves to show the change of meniscus reading corresponding to the change in can volume. The inside diameter of the glass tube is 10 mm. The can is observable across the plexiglass cover of the chamber.

The symbolic equation $\Delta V = f(\Delta p)$ will be used several times hereafter to indicate the relation between load (difference in pressure) and deformation (change of volume). The independent variable is Δp because this can be varied in the course of measurements and can be set at predetermined values.

1.3. Measurement (Type A) of the relation between deformation and load by a single loading cycle

In this case the loading of the can placed in the measuring instrument as described in para. 1.2. begins with zero overpressure, hereafter increasing it evenly to the maximum value, then decreasing it again evenly to zero. Hereafter, the vacuum is increased to a maximum value. Next the vacuum is decreased to zero. To each pressure difference the pertinent change of volume is read on the meniscus. This type of measurement was used with experiments of serial numbers 1 to 5 and illustrated in Fig. 2 and Fig. 4.

1.4. Measurement (Type B) of the relation between deformation and load by multiple loading cycles

In this case, too, the loading of the can begins at zero overpressure, then the vacuum is increased by 0.1 bar and reduced again to zero overpressure. Hereafter the value of vacuum is increased to 0.1, then to 0.2 bar and then reduced again to zero with differences of 0.1 bar and so on. The value of the maximum vacuum is 0.5 bar, i.e. the pressure difference is -0.5 bar. This measurement phase is called "Vacuum I".

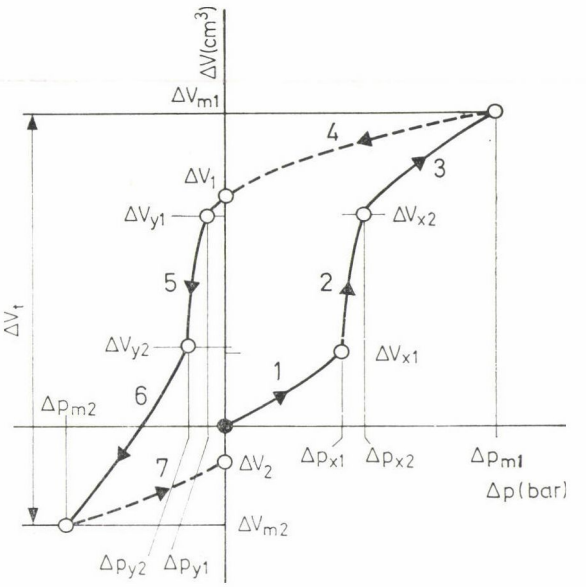


Fig. 2. Characteristic $\Delta V = f(\Delta p)$ relation in the case of a single load cycle (A-type experiment). 1: curve of initial can dilatation; 2: curve illustrating the sudden bulging of the end plates; 3: curve illustrating further dilatation at the end plates; 4: returning branch; 5: curve pertaining to sudden indentation of the end plates; 6: further indentation of the end plates; 7: phase of final relase of load

In the next phase of measuring the same occurs, however, up to 1 bar overpressure i.e. positive pressure difference. This measurement phase is denominated "Overpressure".

In the third phase of measuring the same happens as in phase I (Vacuum I) with the difference that up to the maximum value of 0.6 bar vacuum. This phase is denominated "Vacuum II".

Figure 3 illustrates the course of measurement. This Type B measurement was applied in the experiments of serial numbers 6, 7 and 8.

1.5. Measurements to determine the critical pressure differences

The critical pressure difference caused by internal overpressure (Δp_{KRt}) is a pressure difference of positive value resulting in a well-marked (obtrusive) permanent deformation of the end plates. This deformation remains even after the reduction of pressure difference to zero. It has to be noted, however, that some partial permanent deformation may occur at a pressure difference much lower than the critical pressure difference. From the point-of-view of industrial practice it is important to know the value of Δp_{KRt} because if during heat treatment this value is reached or exceeded the canned product is not suitable for the market (Type C measurement).

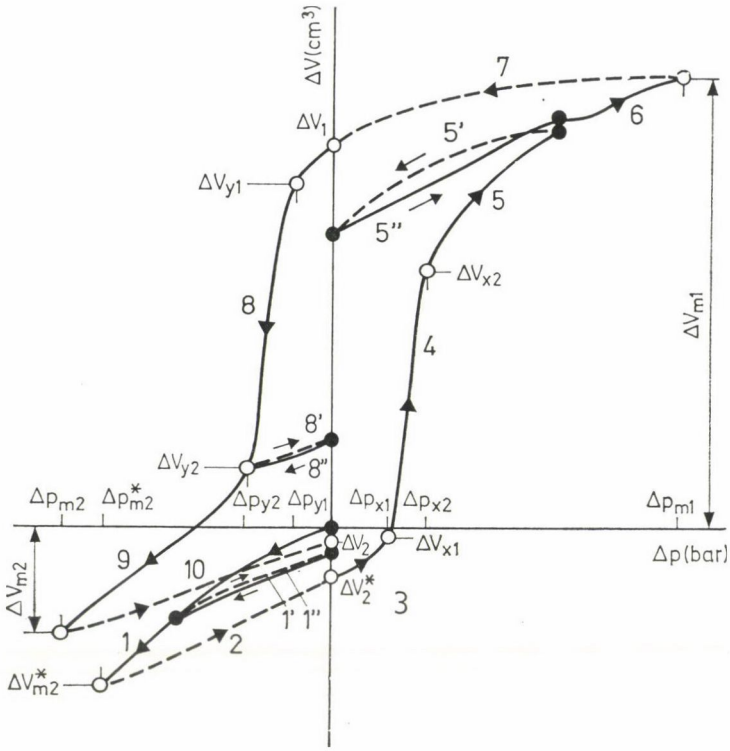


Fig. 3. Characteristic $\Delta V = f(\Delta p)$ relation in the case of multiple load cycles (B-type experiments). 1: curve illustrating the initial formation of vacuum; 1': return to $\Delta p = 0$; 1": repeated increase of depression; 2: final return to $\Delta p = 0$; 3: curve of the initial inner overpressure; 4: curve of sudden bulging; 5: deformation of the end plates; 5': and 5": similar to curves 1' and 1"; 6: continuation of curve 5; 7: returning branch; 8: curve illustrating sudden indentation; 8: curve of further indentation of end plates; 10: phase of final release of load. Solid line (——): absolute value of pressure difference increasing; dashed line (– – –): absolute value of pressure difference decreasing

The critical pressure difference caused by external overpressure (Δp_{KRv}) is a pressure difference of negative value in consequence of which the jacket is staved in. The collapse of the can is initiated by a suddenly applied (small) force perpendicular to the surface. To attain this result in the measurements the surface of the can was pressed with two fingers (Type D) measurement.

In establishing the two critical loads the chamber (marked 3) as well as the cover (marked 1) of the measuring instrument were removed (Fig. 1).

1.6. Methods of evaluation

The following evaluation methods were applied: calculation of the mean and standard deviation (estimated dispersion), one-way analysis of variance, t, F and Bartlett's Chi square test. Type EMG 666 B programmable calculator

and the EMG 839 matrix printer were used in analyses of variance, the Bartlett test and calculation of the mean and scatter.

In the mathematical statistical calculations the measured values of changes in volume (ΔV) were taken into account because the pressure differences were preset (Δp).

2. Results

2.1. Results of Type A measurements

Measurements of Type A were applied in the experiments of serial numbers 1 to 5. Results of the experiments have been already published by FERENCZY and KÖRMENDY (1984).

In the summarized evaluation of the 8 experiments only those data were taken into account where the absolute value of pressure difference is the integer multiple of 0.1 bar.

In plotting the points of measurements diagrams similar to the curve as seen in Fig. 2 were obtained. As an example the results of the experiment with serial number 5 are shown in Fig. 4.

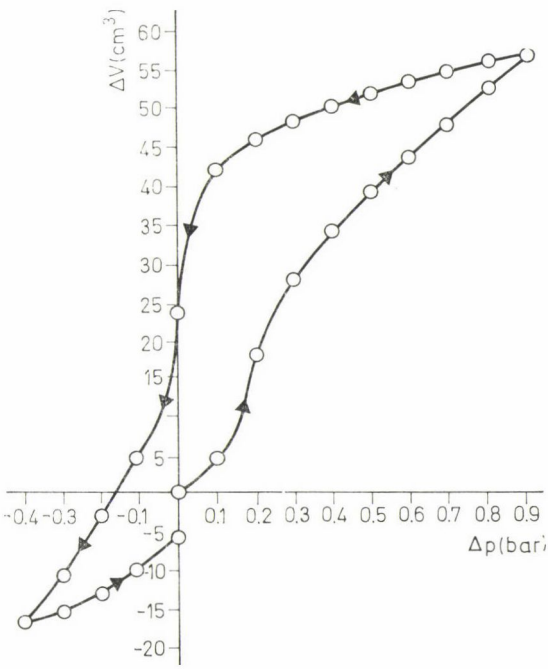


Fig. 4. Relation $\Delta V = f(\Delta p)$ illustrated with the repetitive means of experiment No. 5

2.2. Results of Type B measurements

B-type measurements were applied to experiments of serial number 6, 7 and 8. The average values of volume changes, similarly to Fig. 5 are shown in Figs. 6, 7 and 8 as a function of pressure differences. Pressure differences, here too, are preset values.

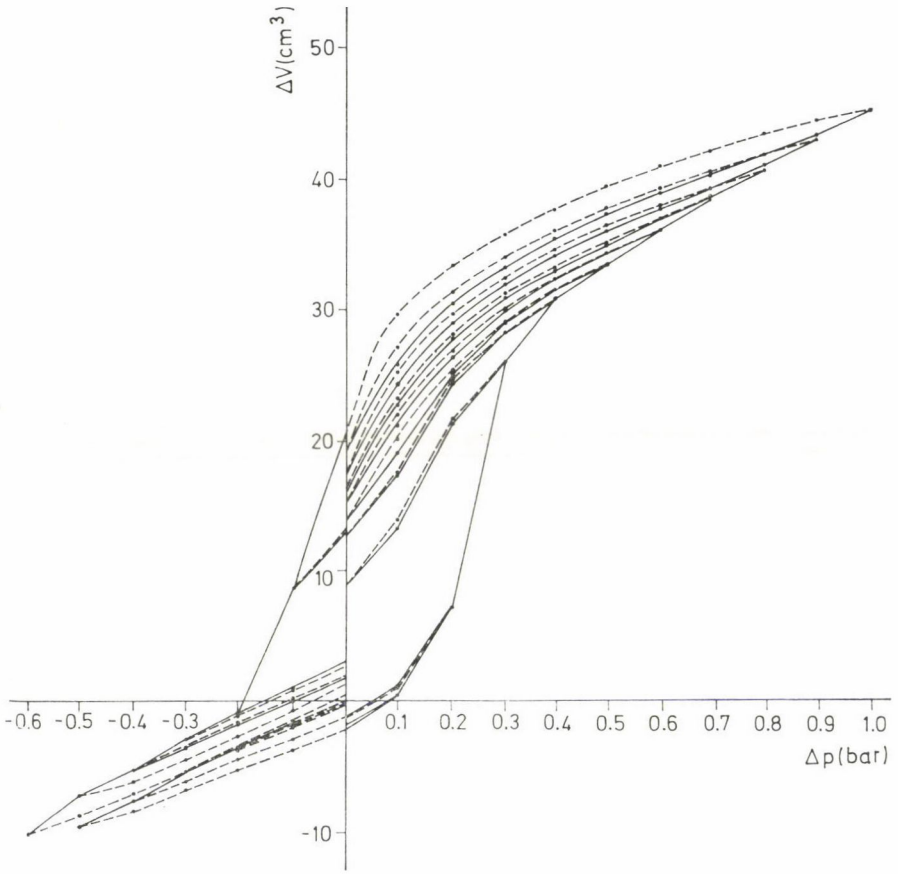


Fig. 5. Relation $\Delta V = f(\Delta p)$ from experiment No. 6. ΔV is the average change of volume from three repeated experiments. Nominal thickness of end plates: 0.24 mm. Solid line (——): the absolute value of pressure difference increases; dashed line (– – –): the absolute value of pressure difference decreases

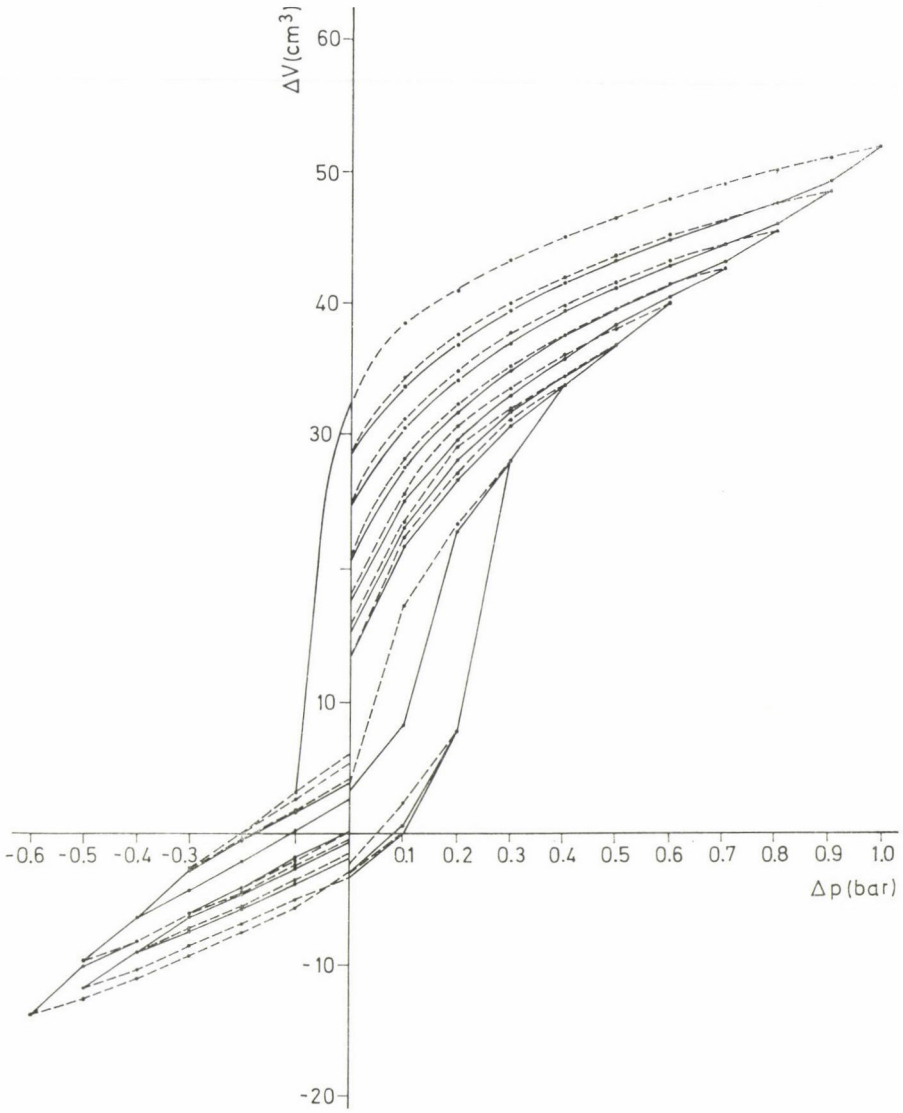


Fig. 6. Relation $\Delta V = f(\Delta p)$ from experiment No. 7. ΔV represents the average of volume change from three repetitions. Nominal thickness of the end plate: 0.22 mm. Solid line (——): absolute value of pressure differences increasing; dashed line (– – –): absolute value of pressure differences decreasing

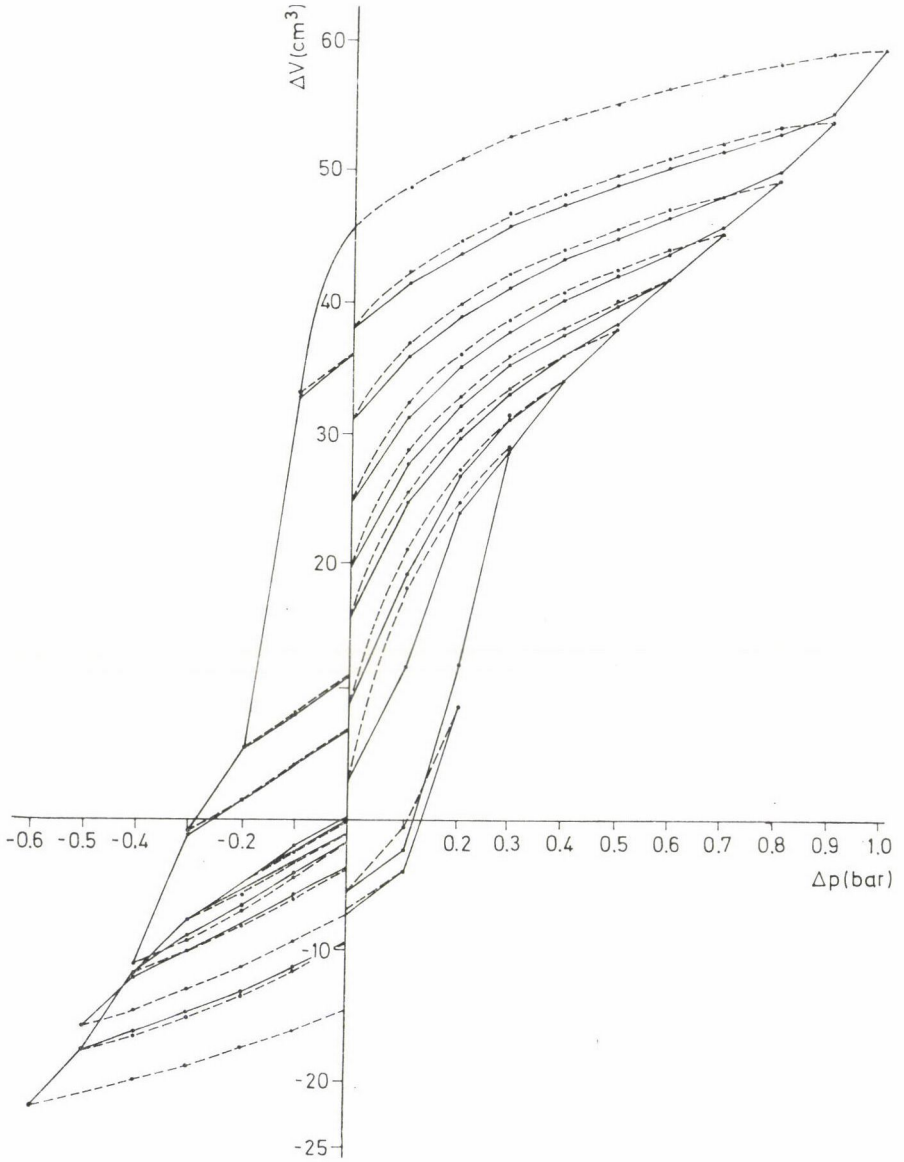


Fig. 7. Relation $\Delta V = f(\Delta p)$ from experiment No. 8. ΔV represents the average of volume change from three repetitions. Nominal thickness of end plate: 0.20 mm. Solid line (——): absolute value of pressure differences increasing; dashed line (– – –): absolute value of pressure differences decreasing

2.3. Results of Type C and D measurements

These results are shown in Table 3.

Table 3

Critical pressure differences related to cans marked ∅ 99–1/1

Serial number of experiment	Nominal thickness of plate (mm)		Mean value of Δp_{KRt} (bar)	Mean value of Δp_{KRv} (bar)	n
	jacket	end plate			
1	0.24	0.24	1.593	−0.670	6
2	0.22	0.24	1.593	−0.647	6
3	0.22	0.22	1.377	−0.600	6
4	0.20	0.20	1.227	−0.540	6
5	0.18	0.18	1.033	−0.517[a]	6
Standard deviation (bar) D. F. = 10			0.0563	0.0036	–

n: number of indentations of the jacket
[a]: at −0.52 bar pressure difference the jacket staves in without any outside intervention

3. Evaluation of the results of measurements and conclusions

3.1. Evaluation of results and conclusions of Type A experiments

The results of Type A experiments gave the curve as seen in Fig. 2. The most expedient course is to characterize such a curve by the (Δp, ΔV) pairs of values belonging to the 9 characteristic nodes in the figure. It is also necessary to display the arrows showing unambigously the course of loading. The characteristic values obtained from experiments 1 to 5 have been already published (FERENCZY & KÖRMENDY, 1984).

It is of particular interest that ΔV_2, the volume change at the termination of the load cycle, is generally, but not always below zero. This is supported also by the measurements of DELI (1982). In the $\Delta V = f(\Delta p)$ diagrams shown in the studies of HEISS (1955), as well as of ROGACSEV and BABARIN (1983), $\Delta V_2 = 0$.

The case $\Delta V_2 \neq 0$ shows that the applied load induced already some permanent deformation. The case $\Delta V_2 = 0$, however, can be explained so that the can suffered essentially only elastic deformation and at the end of the measuring cycle it recaptured its original shape and volume.

If in the course of parallel measurements, as it occured during experiments No. 1 and No. 2, ΔV_2 assumes low positive or negative values, then it can be presumed:

Slight permanent deformations have occurred where the end plates join the jacket, in the course of deformation of the end plates not exactly known slight effects cause fluctuation around $\Delta V_2 = 0$.

The measurements have shown that the can types (surface not undulated) used changed their volume mostly in consequence of displacement of the end plates. This was concluded from the fact that the results of experiments 1 and 2 did not produce significant differences related to $\overline{\Delta V}_{m1}$ (the average of the parallel measurements of ΔV_{m1}).

This was proven also by calculations: change in the volume of such a cylinder, if the boundary surfaces are not deformed gives only about 0.5% of the total change of volume. This conclusion is not valid for cans of undulated jacket surface. More detailed information can be found in the papers of FERENCZY and KÖRMENDY (1984) and FERENCZY (1986).

On comparing the results of the experiments of serial numbers 1 to 5, it can be concluded that in the case of end plates of the same hardness category the absolute value of the volume change belonging to the same pressure difference increases with the reduction of plate thickness. When the hardness of the end plates differed, the former relation changed adequately (FERENCZY & KÖRMENDY, 1984).

Experiments of Type A are suitable for the comparison of cans made of plates of different thickness and quality, but otherwise of identical type. In this case, however, always identical values have to be selected for Δp_{m1} and Δp_{m2}. The part marked 1, 2 and 3 of the relation $\Delta V = f(\Delta p)$ as shown in Fig. 2 is called guiding curve. The further course of the diagram depends on the Δp_{m1} value at which the reduction of pressure begins, that is it branches back from the guiding curve.

On comparing with experiments Type B it may be seen that this is a special guiding curve, a guiding curve without initial depression or initial negative pressure difference. This type is characteristic of every industrial heat treatment, where in the warming up phase the internal pressure in the can increases faster than the ambient external pressure.

3.2. Evaluation of results and conclusions of experiments Type B

Results of the experiments are illustrated in Figs. 5, 6 and 7. The experimental process is described in paras 1.4 and 2.2.

Measurements begin with phase "Vacuum I". This way of loading tries to imitate the industrial process where sealing under vacuum is applied or vacuum is formed in the sealed can during cooling. The curves belonging to phase "Vacuum I" fall in the space section $\Delta p < 0$, $\Delta V < 0$. Increase of load or of the absolute value of pressure difference, as in other phases, is marked by a solid line. Reduction of load or reduction of the absolute value of pressure

difference is marked by a dashed line. Phase "Vacuum I" is characterized by the guiding curve of initial depression as it appears in Fig. 3 (curve marked 1).

The decrease of volume belonging to monotonously increasing vacuum values can be seen on this guiding curve. Upon decrease of the vacuum the reversed curves marked 1', ... 2 branch off the guiding curve. The ΔV_2^* value belonging to $\Delta p = 0$ marks the starting point of the overpressure guiding curve as modified by the initial vacuum.

It is also worthy of attention that after reducing the vacuum and then increasing it again from $\Delta p = 0$, we get back practically along the former curve to the branching off point, thus the solid and dashed curves cover one another (curves marked 1' and 1'').

The phase "Overpressure" starts at $\Delta p = 0$ and $\Delta V_2^* < 0$ value. The value of the latter depends on the highest vacuum in phase "Vacuum I". With a monotonously increasing internal overpressure the change of volume, here too, follows the guiding curve (curves 3, 4, 5 and 6 in Fig. 3). This guiding curve, however, is displaced in the direction of lower change of volume as compared to the guiding curve of the Type A experiment (see Fig. 2). Here, too, the lines belonging to diminishing pressure differences branch off the guiding curve (curves marked 5', ... 7 in Fig. 3). On increasing the pressure difference again we get back near to the branching off point, but the differences between the deformations belonging to decreasing and increasing pressure differences are somewhat greater than in the phase "Vacuum I". The difference between the related dashed and solid curves is perceptible. The sudden bulging of the end plates is also observable here, as with experiments Type A.

In the next phase, "Vacuum II" occurs the abrupt indentation of the end plates, thus, the change of volume is here substantially more extensive than in phase "Vacuum I". Otherwise results can be similarly explained as in the preceding phase. One can speak here, too, of a guiding curve, the guiding curve following the abrupt indentation (curve marked 9 in Fig. 3), curves belonging to diminishing vacuum values branch off from this (e.g. curve 10 in Fig. 3).

The characteristic values belonging to experiments 6, 7 and 8 are given in Table 4. The notations of this Table can be found in Figs. 2 and 3.

3.3. Evaluation of results and conclusions of experiments Type C and D

Results of experiments belonging here are contained in Table 3. The critical values must never be exceeded, neither in industrial practice, nor in the measurements of Type A and B.

It is obvious that the critical loads (their absolute values) decrease with decreasing plate thickness. The number of indentations from staving in on the jacket was consistently 6, which is in agreement with calculations carried out on the basis of mechanical shell and membrane theory but here not detailed.

Table 4

Characteristic values of the cans based on Type B experiments. Average of parallels are

Serial number of experiment	Nominal thickness of plate jacket/ end plate	Δp_{m1}	ΔV_{m1}	Δp_{m2}	ΔV_{m2}	ΔV_1	ΔV_2	Δp_{x1}	Δp_{x2}
6	0.2/0.24	1	45.7	−0.6	−10.5	20.47	0.25	0.2	0.3
7	0.2/0.22	1	52.43	−0.6	−13.93	32.27	−3.13	0.2	0.3
8	0.2/0.2	1	59.9	−0.6	−22.45	45.8	−14.75	0.1	0.3

\# No characteristic curve for sudden indentation has been obtained (see Fig. 6)

The critical value of the jacket's collapse is not indicated by the relation $\Delta V = f(\Delta p)$. However, prior to the critical value of internal overpressure the guiding curve becomes more and more steep, due to plastic deformation. This observation may form the basis for the more exact measurement of the critical value of the internal overpressure, or for the determination of the extent of the safety zone.

To study the collapse of the can the application of a well defined spring force instead of pressing with two fingers, will be necessary.

3.4. Summarized evaluation of the results of experiments Type A, B, C and D and conclusions

It is shown by the results that in relation to a given type of can the related values of load and deformation fall within or on the border of a well defined region establishable by measurement. The region is bordered by the modified overpressure guiding curve belonging to the industrially possible highest initial vacuum (Δp^*_{m2}), by the reverse curve belonging to the maximum applicable overpressure (Δp_{m1}), then again by the guiding curve following sudden indentation heading towards the highest value of vacuum (Δp_{m2}), followed by the curve reverting to $\Delta p = 0$, or by the reverse curve branching back from the guiding curve of the initial depression. The latter when it leads under the former. It may occur that curves marked 4, 5 and 6 in Fig. 2 lead above the curves marked 7, 8 and 9 in Fig. 3. In this case, differently from the aforesaid, the region is bordered by curves marked 4, 5 and 6 in Fig. 2.

3.4.1. Examination of the variances. The variance is understood to be the square of the standard deviation of volume change (ΔV) belonging to fixed (preset) pressure difference in case experiments were carried out identically with cans having end plates (upper and lower) of the same quality and thickness.

Sources of the variance:

— Fluctuations in manufacture (quality of plate, can making, sealing);

presented in the table $[\Delta p] = bar$, $[\Delta V] = cm^3$

Serial number of experiment	Nominal thickness of plate jacket/ end plate	ΔV_{x1}	ΔV_{x2}	Δp_{y1}	Δp_{y2}	ΔV_{y1}	ΔV_{y2}	ΔV_2^*	Δp_{m2}^*	ΔV_{m2}^*
6	0.2/0.24	7.3	26.2	#	#	#	#	−2.0	−0.5	— 9.7
7	0.2/0.22	7.8	28.1	0.0	−0.1	32.27	3.23	−3.33	−0.5	−11.9
8	0.2/0.2	−2.2	29.43	−0.05	−0.2	39.4	5.25	−7.33	−0.5	−16.4

Table 5

Table of variances of parallel measurements

Serial number of experiment, nomination of the measurement phase		Type of can: thickness of jacket and of end plate (mm)		Degrees freedom (D. F.)	Sum of squares of deviations (S, cm^6)	Square of standard deviation (s^2, cm^6)	Standard deviation (s, cm^3)
1–2	1.1 Overpressure			100	148.261024	1.4826	1.2176
	1.2 Vacuum	0.24	0.24	48	324.721500	6.7650	2.6010
	1.3 Total	0.22	0.24	148	472.982524	3.1958	1.7870
3	3.1 Overpressure			25	39.855766	1.5942	1.2626
	3.2 Vacuum	0.22	0.22	14	20.525000	1.4661	1.2108
	3.3 Total			39	60.380766	1.5482	1.2443
4	4.1 Overpressure			50	216.487182	4.3297	2.0808
	4.2 Vacuum	0.20	0.20	18	55.321667	3.0734	1.7531
	4.3 Total			68	271.808849	3.9972	1.9993
5	5.1 Overpressure			19	1.091787	0.0575	0.2398
	5.2 Vacuum	0.18	0.18	8	58.175255	7.2719	2.6966
	5.3 Total			27	59.267043	2.1951	1.4816
6	6.1 Vacuum I			62	17.787091	0.2869	0.5356
	6.2 Overpressure	0.24	0.24	220	1740.626666	7.9119	2.8128
	6.3 Vacuum II			73	762.145000	10.4403	3.2312
	6.4 Total			355	2520.559378	7.1002	2.6646
7	7.1 Vacuum I			62	144.181536	2.3255	1.5250
	7.2 Overpressure	0.22	0.22	220	2939.226666	13.3601	3.6552
	7.3 Vacuum II			86	689.058333	8.0123	2.8306
	7.4 Total			368	3772.246562	10.2507	3.2017
8	8.1 Vacuum I			62	1.796407	0.0290	0.1702
	8.2 Overpressure	0.20	0.20	209	303.815845	1.4537	1.2057
	8.3 Vacuum II			44	389.190511	8.8452	2.9741
	8.4 Total			315	695.240778	2.2071	1.4856

— error in reading the change of volume. Error of reading originating in the standard deviation of reading the meniscus and this amounted to 0.016 cm^3;

— error in reading the pressure difference. Since preset pressure differences were applied the standard deviation in measuring the pressure difference is increasing the standard deviation of the change of volume:

$$s_p(\Delta V) = s(\Delta p) \frac{d\Delta V}{d\Delta p};$$

— other errors of measurement (setting the apparatus, temperature, etc.).

In the investigations experiments 1 and 2 were contracted (end plates of identical thickness) thus, altogether 7 variances from parallels were analysed. The main data and results are presented in Tables 5 to 10.

Table 6

Table of variances from parallels regarding experimental groups

Denomination of group	Degrees of freedom (D. F.)	Sum of squares of deviations (S, cm^6)	Square of standard deviation (s^2, cm^6)	Standard deviation (s, cm^3)
Experiments Nos. 1 to 5	282	864.439182	3.0654	1.7508
Experiments Nos. 6 to 8	1038	6988.046718	6.7322	2.5946
Experiments Nos. 1 to 8	1320	7852.485900	5.9488	2.4390

Table 7

Bartlett's test of homogeneity of variances within individual experimental phases

Serial number of experiment, denomination of the phase		Number of groups of parallels	Calculated Chi-square	Tabulated Chi-square	Judgement[a]
1–2.	1.1	25	65.47	36.15	not homogeneous
	1.2	14	2.31	22.36	homogeneous
3.	3.1	25	19.08	36.15	homogeneous
	3.2	14	1.58	22.36	homogeneous
4.	4.1	25	32.99	36.15	homogeneous
	4.2	12	3.61	19.68	homogeneous
5.	5.1	19	28.34	28.87	homogeneous
	5.2	8	16.16	14.07	not homogeneous
6.	6.1	31	33.94	43.77	homogeneous
	6.2	110	132.14	134.09	homogeneous
	6.3	43	76.93	57.84	not homogeneous
7.	7.1	31	70.89	43.77	not homogeneous
	7.2	110	37.79	134.09	homogeneous
	7.3	44	42.77	59.02	homogeneous
8.	8.1	31	65.86	43.77	not homogeneous
	8.2	110	306.96	134.09	not homogeneous
	8.3	44	153.69	59.02	not homogeneous

[a] see Table 8

Table 8

Comparison of the average variances of experiments 1 to 5 or their phases by Bartlett's test

Name and notation of phase	Calculated chi-square	Tabulated chi-square	Judgement[a]	Average of the square of standard deviation (s^2, cm^2)	Average of standard deviation (s, cm^3)
Overpressure					
1.1					
3.1	72.14		not homogeneous	2.0912	1.4461
4.1					
5.1					
Vacuum					
1.2					
3.2	11.76	7.82	not homogeneous	5.2130	2.2832
4.2					
5.2					
Total					
1.1–1.2					
3.1–3.2	11.34		not homogeneous	3.0654	1.7510
4.1–4.2					
5.1–5.2					

[a] in case Chi-square calculated is < Chi-square tabulated variances are homogeneous, if Chi-square calculated is > Chi-square tabulated variances are inhomogeneous. The same applies to the F value, too

Table 9

Comparison of the average variances relating to experiments 6 to 8 or their phases by Bartlett's test

Name and notation of phase	Calculated Chi-square	Tabulated Chi-square	Judgement[a]	Average of the square of standard deviation (s^2, cm^2)	Average of standard deviation (s, cm^3)
Vacuum I					
6.1					
7.1	219.40		not homogeneous	0.8805	0.9384
8.1					
Overpressure					
6.2					
7.2	219.00	5.99	not homogeneous	7.6790	2.7711
8.2					
Vacuum II					
6.3					
7.3	1.4		homogeneous	9.0660	3.0110
8.2					
Total					
6.1–6.2–6.3					
7.1–7.2–7.3	177.45		not homogeneous	6.7322	2.5946
8.1–8.2–8.3					

[a] see Table 8

Table 10

Results of Bartlett and F *tests of the variances related to phases within individual experiments*

Serial number of the experiment and notation of phase	Calculated Chi-square or F value	Tabulated Chi-square, or F value	Judgement[a]
1–2 1.1	$F = 4.56$	1.48	not homogeneous
1.2			
3 3.1	$F = 1.09$	2.34	homogeneous
4 4.1			
4.2	$F = 1.41$	1.86	homogeneous
5 5.1			
5.2	$F = 126.47$	2.48	not homogeneous
6 6.1			
6.2	Chi-square =		not homogeneous
6.3	146.21		
7 7.1			
7.2	Chi-square =	5.99	
7.3	54.63		not homogeneous
8 8.1			
8.2	Chi-square =		
8.3	292.67		not homogeneous

[a] see Table 8

The number of parallels varied between 2 and 5. The results were analysed in view of the following questions:

a) Can variances be considered homogeneous regarding an experiment of given serial number and a definite phase of it?

b) Can the variances of the overpressure or vacuum phases of the first five experiments (serial number 1 to 5) or the average variances of the two be considered homogeneous?

c) In relation to the variances of the last three experiments (serial No. 6 to 8) practically the same question is raised as under the foregoing point *b*).

d) Can the average variances of the phases of any of the experiments be considered homogeneous?

e) Can the average variances of the eight experiments be considered homogeneous?

Two variances were compared by F test, more than two variances by Bartlett's test. The null hypothesis for both tests is that variances are derived from populations of identical dispersion (but not necessarily of identical expectation values). For both tests in case of rejecting the null hypothesis 5% error probability was selected ($\alpha = 0.05$). In the Bartlett test the standard deviations calculated as zero were replaced by the standard deviation of reading ($s_1 = 0.016$ cm^3).

The results related to question *a)* are presented in Table 7. As seen from the table out of 17 phases in 7 the variances were not homogeneous.

The results on question *b)* are presented in Table 8. As shown in the table average variances were not homogeneous in any of the cases.

The answer to question *c)*, as seen in Table 9, is similar except for the second phase of measurement under vacuum ("Vacuum II", phases 6.3, 7.3 and 8.3).

The answer to question *d)* on the basis of Table 10 is as follows: the average variances of two of the experiments (No. 3 and No. 4) were homogeneous. The individual phases here had also homogeneous variances (see Table 7).

The answer to question *e)*: the average variances of the experiments are not homogeneous. The calculated Chi-square is 246, the tabulated Chi-square is 12.59. The average variance from parallels of all the experiments is 5.95 cm^6, the average standard deviation: 2.44 cm^3 (see Tables 5 and 6). The average variances of the individual experiments fluctuated between 1.55 and 10.25 cm^6.

The average variances of the experimental phases fluctuated between 0.03 and 13.36 cm^6 with average standard deviations of 0.17 and 3.66 cm^3. The analyses can be utilized in calculating the internal pressures or pressure differences developing during sterilization, for comparing the measured and calculated values.

The results here presented are suitable to deliberate the reduction of plate thickness and to calculate the industrial loads of the cans by incorporating them into known methods of calculation (KÖRMENDY, 1982).

Literature

DELI, L. (1982): *A belső nyomás mérése és számítása konzervdobozokban.* (Measurement and calculation of internal pressure in cans.) Diploma work. Kertészeti és Élelmiszeripari Egyetem, Budapest.

FERENCZY, I. (1986): *Konzervdoboz deformációjának mérése és a hazai dobozgyártás fejlesztése.* (Measurement of the deformation of cans and the development of can manufacture in Hungary.) Doctoral thesis. Kertészeti és Élelmiszeripari Egyetem, Budapest.

FERENCZY, I. & KÖRMENDY, I. (1984): Konzervdobozok deformációjának mérése az anyagtakarékosság érdekében. (Measurement of the deformation of cans for material saving.) *Élelm. Ipar, 38*, 173–180.

HEISS, R. (1955): *Fortschritte in der Technologie des Konservierens von Gemüse und Obst.* Verlag und Druck Dr. Serger und Hempel, Braunschweig.

HUNGARIAN STANDARD (1980): *Konzervdobozok.* (Cans for preservation.) MSZ-08 1467–80.

KÖRMENDY, I. (1982): *A hőkezeléssel történő tartósítás korszerű ipari technológiái.* (Modern industrial technology of canning by heat treatment.) Lecture notes. Kertészeti és Élelmiszeripari Egyetem, Budapest.

KÖRMENDY, I. (1987): Outline of a system for the selection of the optimum sterilization process for canned foods. *Acta Alimentaria, 16*, 3–27.

ROGACSEV, V. I. & BABARIN, V. P. (1983): *A konzervipar folyamatos hőkezelő berendezései.* (Continuous heat treatment equipments in the canning industry.) Mezőgazdasági Kiadó, Budapest, pp. 50–62.

Acta Alimentaria, Vol. 18 (4), pp. 353–360 (1989)

PREPARATION AND FUNCTIONAL PROPERTIES OF ACETYLATED RAPESEED PROTEINS

S. Gwiazda, K. J. Dabrowski, M. Klepacka and A. Rutkowski

Agricultural University of Warsaw, Department of Food Technology, Grochowska 272, 03-849 Warszawa. Poland

(Received: 24 March 1988; revision received: 7 February 1989; accepted: 8 February 1989)

The protein isolates from rapeseed flour of traditional and improved varieties were prepared by the conventional method and the process involving the use of acetic anhydride during extraction followed by isoelectric precipitation of the proteins. The effects of acetylation on the protein extractability, isoelectric precipitation and functional properties of the protein isolates including examination in the mayonnaise and margarine model systems were studied. Acetylation (91% modification of free amino groups) markedly increased protein extraction and precipitation yield which consequently gave 45% increase of protein isolation yield. At the same time the phytic P content was reduced by about 95%. Some functional properties of acetylated protein isolates were also superior over the unmodified samples. Especially, nitrogen solubility, water absorption capacity, emulsifying properties as well as the colour of resulting isolates were improved.

Keywords: rapeseed proteins, acetylation, functional properties

Despite very well balanced amino acid composition, rapeseed protein has not so far found application as a food component. It is mainly due to low yield of the protein isolation process affected by both native properties of rapeseed proteins (Rutkowski & Gwiazda, 1986) and necessity of removal of undesirable compounds i.e. glucosinolates, phenolics, phytates. Introduction of double improved rapeseed (low erucic acid and low glucosinolate contents) to the commercial scale of production, in general has not changed its usefulness as a raw material for preparation of the edible protein products. Therefore further searching of technological solutions is still observed. These new technological approaches should, beside increasing the yield of the protein isolation process, enable to obtain protein products having specific functional properties. In this case high cost of the protein preparation would be justified by gaining a final product of high quality. Among the techniques that have been developed in recent years some expectations may be linked to chemical modification (Feeney et al., 1982), especially to acetylation of rapeseed proteins as recommended by Thompson and Cho (1984a, b).

The aim of the present study was to obtain and evaluate the functional properties of acetylated proteins from traditional and improved rapeseed.

1. Materials and methods

1.1. Materials

Hexane extracted rapeseed flours (RF) were prepared in the laboratory from:

— traditional (T),
— low erucic acid (LE), and
— double low (low erucic acid and low glucosinolate; DL) rapeseed of Gorczanski, JG-75 and MAH variety, respectively.

1.2. Sample preparation

Defatted RF was dispersed in distilled water (1 : 10, w/v) and during extraction of proteins at pH 8.5 for 60 min at room temperature, simultaneous acetylation has been carried out by addition drop-wise of acetic anhydride in amount of 0.186 $cm^3 g^{-1}$ of protein (THOMPSON & CHO, 1984b). Under these conditions, 91.6% of the protein has been acetylated as analysed by Carpenter method (BOOTH, 1971) of available lysine determination that was conducted by courtesy of Prof. K. D. Schwenke, Central Institute of Nutrition, GDR. After centrifugation (12 000 × g) proteins from the extracts were precipitated by addition of 5 *N* HCl to reach pH 3.5 at which the highest yield of precipitation was found. Then the resulting precipitates were washed with distilled water (1 : 5, w/w) at pH 3.5 redispersed in water, adjusted to pH 7.0 and spray-dried. The drying was carried out with Niro Atomizer spray dryer at inlet and outlet temperatures of 165 °C and 80 °C, respectively.

Parallel, unmodified (control) sample was prepared by the conventional method using extraction and precipitation at pH 9.5 and pH 4.5, respectively. In the course of protein preparation processing, the solid samples, protein extracts and supernatants were weighed and analysed for nitrogen content in order to determine the extraction and isolation yields.

1.3. Methods

Crude protein, water, total phosphorous contents were determined in triplicate by the standard procedures. Phytic phosphorous content was analysed after precipitation of ferrum phytate, mineralization and colorimetric determination (OBERLEAS, 1971). Functional properties of the obtained rapeseed protein isolates (RPI) were evaluated by the measurement of soluble nitrogen at pH 3.5, 5.5 and 7.0; water absorption capacity, emulsifying activity and emulsion stability, foam expansion and foam stability (GWIAZDA & KOCON,

1979) as well as by the examination in mayonnaise and margarine model systems.

The low fat mayonnaise sauces (30% oil) were prepared using the following ingredients: soybean oil, milk powder, yolk powder, Frimulsion stabilizer, NaCl, vinegar, water and RPI of 0 and 0.4%. The mayonnaise samples were prepared by mixing all ingredients (with the exception of oil and vinegar) for 1 min using homogenizer type 309. After 5 min of quiescent period hot soybean oil (80 °C) was added under continuous mixing during 2 min and at the final stage vinegar was added. In the obtained mayonnaise model samples weight losses under samples centrifugation (4000 r.p.m. 5 min) and freezing (—20 °C, 48 h) were evaluated.

The samples of margarine model system were prepared using following ingredients: hydrogenated rapeseed oil (49.0%), soybean oil (21.0%), water (29.5%), ME emulsifier (0.3 and 0.45%), lecithin (0 and 0.05%) and RPI (0 and 0.25%). Preparation process was as follows: the aqueous phase containing RPI has been added to liquid fat phase which was brought to 40 °C and emulsified for 2 min using homogenizer type 302 in order to obtain homogeneous emulsion. The aluminium container with the emulsion was placed in a cold brine bath mixed with low speed homogenizer type 302 until solid emulsion has been formed. After 24 h of storage in the refrigerator (5 °C) in the obtained samples of margarine weight losses during frying under standard conditions and dispersion degree of aqueous phase according to ZSCHALER (1977) were evaluated.

Colour of dry RPI has been determined with MOMCOLOR D photocolorimeter (MOM, Hungary and expressed as the lightness value (Y_{CIE}).

2. Results and discussion

Results on yield of nitrogen substances in the course of RPI preparation are shown in Table 1. Low extractability of nitrogen (58%) using the conventional method of RPI has been increased to 69% when the acetylation process was used. It proves that acetylation applied during protein extraction may not only modify properties but also increase the yield of the extraction process which was however lower than described by THOMPSON and CHO (1984a) for a similar degree of protein acetylation (91% vs 87%). Precipitation yield of proteins from the raw extracts was also found higher when acetylation was used (Table 1). As a result, considerably higher i.e. about 45% of yield of the nitrogen substances in form of the final isolate has been obtained. In comparison to other methods of RPI preparation e.g. those of EL NOCKRASHY and co-workers (1977), THOMPSON and co-workers (1982a) the yield obtained in this study is considered low. However, it should be underlined that contrary to

Table 1

Nitrogen yield (%) in the course of preparation of control and acetylated rapeseed protein isolates[a]

Course of preparation	Control (RPI-LE)	Acetylated		
		RPI-LE	RPI-DL	RPI-T
Extraction (g N extracted per 100 g N in rapeseed flour)	57.8	69.4	65.8	60.1
Precipitation (g N precipitated per 100 g N extracted)	46.0	54.5	58.1	45.0
Isolation (g N isolated per 100 g N in rapeseed flour)	26.6	37.8	38.2	27.0

[a] Data are the mean values of duplicate run
Symbols: RPI: rapeseed protein isolate; LE: low erucic acid rapeseed; DL: double low rapeseed; T: traditional rapeseed

above cited results, in the present study only one-stage extraction and precipitation process was used. Thus, further increase of nitrogen yield is still possible. Among three different rapeseed varieties examined in this study higher yields were obtained of both, nitrogen extraction and precipitation processes from improved rapeseed (LE, DL) (Table 1). It suggests somewhat better technological usefulness of both evaluated improved rapeseed cultivars for RPI preparation by this technique than that one of traditional rapeseed (T). Similar variability in the protein extraction and precipitation yield from various cultivars of rapeseed have been already shown by EL NOCKRASHY and co-workers (1977) and THOMPSON and co-workers (1982a).

Table 2

Water, protein and phosphorous contents in control and acetylated rapeseed protein isolates[a]

Constituent (%)	Control (RPI-LE)	Acetylated		
		RPI-LE	RPI-DL	RPI-T
Water	5.2	5.2	6.6	6.1
Protein (N×6.25)	67.8	64.5	70.8	73.0
Total P:				
RF	1.49	1.46	1.51	1.40
RPI	0.93	0.42	0.37	0.32
Phytic P:				
RF	1.05	1.05	1.15	1.02
RPI	0.40	0.05	0.06	0.06
$\frac{\text{Phytic P}}{\text{Total P}} 100$:				
RF	71.9	71.9	76.1	72.9
RPI	43.0	12.3	16.1	18.3

[a] Data are the mean values of duplicate run
Symbols: RF: rapeseed flour; for other symbols see Table 1

Generally the low protein content in the obtained RPI (Table 2) can be ascribed, among others, to the ineffective process of single washing, applied in this study. Beside that, the acetylation itself decreases protein content in the final isolate (THOMPSON & CHO, 1984b). This study has confirmed possibility of considerable reduction of phytic phosphorous in the final isolate by the acetylation process (Table 2). In the raw material, phytic P amounts to 72–74% of total phosphorous, but in the case of acetylated protein isolates this value was reduced to 12–18%. Decrease of phytic P in acetylated RPI related to the initial value in the flour amounts to about 95%, similarly as shown by CHO and THOMPSON (1984) and THOMPSON and CHO (1984a).

Some functional properties of examined RPI are shown in Table 3. The acetylation process applied at extraction stage has significantly improved nitrogen solubility and water absorption capacity of the resulting acetylated RPI. This is especially important since one of the RPI drawbacks is low nitrogen solubility. Therefore the observed increase of nitrogen solubility caused by acetylation, also at lower pH values, is favourable. This has been earlier observed in the case of other raw materials (FRANZEN & KINSELLA 1976a, b; THOMPSON & CHO 1984b) and is due to increased protein–water interaction as the net negative charges increased upon acetylation. Improvement of emulsifying properties and foam activity of acetylated RPI can also be linked to their better nitrogen solubility (Table 3). However, as CHOI and co-workers (1983) and THOMPSON and CHO (1984b) proved this relationship was not linear. According to NAKAI (1983) emulsifying properties are dependent not only on solubility but also on hydrophile–lipophile balance (HLB) of the protein in question. The acetylation of the proteins may unfold the protein structure and change the HLB toward a more favourable level.

The spray-dried acetylated RPI showed almost 3 times higher water absorption capacity as compared to the unmodified isolate (Table 3) and reached the level obtained in the case of commercial soybean protein isolate. Additionally, the acetylation process significantly improved the colour of the RPI. Similarly to results of FRANZEN and KINSELLA (1976a, b), the acetylated RPI was considerably lighter than the non-modified sample (Y_{CIE} 41 vs 29), while THOMPSON and CHO (1984b) have not found such relationship.

Good emulsifying and absorption properties which acetylated RPI possessed (Table 3) suggest possibility of its application in various food systems where it could play the role of a functional additive. To such products, beside already examined meat systems, beverages and dairy analogues (THOMPSON & RENIERS, 1982; THOMPSON et al., 1982b, 1983) mayonnaise and margarine can be included. Tests conducted in this study with low fat mayonnaise model system have shown that the examined acetylated RPI did not possess as good properties as the Frimulsion stabilizer. However, incorporation of acetylated

Table 3

Functional properties of control and acetylated rapeseed protein isolates[a]

Property	Control RPI-LE	Acetylated			Purine 500 E
		RPI-LE	RPI-DL	RPI-T	
Nitrogen solubility (%):					
pH 3.5	6.0	*12.3*	*4.6*	*3.0*	ND
5.5	5.7	*31.0*	*18.1*	*18.6*	ND
7.0	12.3	*41.0*	*35.0*	*34.6*	ND
Water absorption capacity (g H_2O per g)	4.1	*11.0*	*10.8*	*9.4*	*12.7*
Emulsifying activity (%)	63.5	*85.9*	*91.0*	*90.4*	ND
Emulsion stability (%)	68.1	*89.3*	*93.3*	*92.4*	ND
Foam expansion (cm^3)	128	137	*141*	*139*	*104*
Foam stability (cm^3)	111	*120*	*126*	107	*100*
Colour Y_{CIE}	29.3	*40.6*	30.3	*47.3*	ND

[a]Mean values (n = 4). The values in italics are significantly lower or higher than the control value at $P \leq 0.05$
ND: not determined

RPI in the amount of only 0.4% enables to obtain a model mayonnaise having better emulsion stability than that one without RPI. This is particularly evident in the case of mayonnaise samples subjected to treatments that destroy the structure of the emulsion e.g. freezing (Table 4). Among the RPI-s tested, better properties in mayonnaise model systems have shown the acetylated RPI however, to a lesser extent as it was found using simple tests for determination of physicochemical properties (Table 3).

Experiments with margarine model systems have revealed that examined RPI, to a certain extent, may improve the degree of water dispersion (Table 5),

Table 4

Functional properties of rapeseed protein isolates in mayonnaise type model systems

Sample No.:	1	2	3	4	5	6	7	8	9
Ingredients (%)[a]:									
yolk powder	2.1	2.1	2.1	2.1	2.1	2.1	—	—	—
Frimulsion	0.8	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
RPI (0.4%)	—	control	U-LE	A-LE	A-DL	A-T	control	U-LE	A-LE
Weight losses (%)[b] under:									
centrifugation	0	0	0	0	0	0	0	0	0
freezing and centrifugation	0	2.4	0	0	0	0	9.6	8.6	*6.7*
centrifugation, freezing and centrifugation	0	15.8	*12.1*	*10.8*	*10.6*	*11.1*	26.8	25.4	*20.7*

[a] Basic ingredients: soybean oil (30%), milk powder (3.5%), NaCl (1%), sugar (2.1%), vinegar (3.5%) and water (57%)
[b] cm^3 of separated oil per 100 cm^3 of mayonnaise emulsion; mean values (n = 4). The values in italics are significantly lower or higher than the control values at $P \leq 0.05$
U,A: unmodified and acetylated RPI, respectively

Table 5

Functional properties of rapeseed protein isolates in margarine model systems[a]

Margarine model system (MMS)	Frying losses (%)	Spattering intensity (0–5)	Water phase dispersion (1–8)
Control	34.3	3	3
MMS 1 (0.25% RPI):			
U-LE	*26.5*	*2*	*4*
A-LE	29.7	*2*	*4*
A-DL	*28.0*	*2*	ND
A-T	*27.5*	*2*	ND
MMS 2 (0.25% RPI, no lecithin added):			
U-LE	*42.6*	*4*	ND
A-LE	*48.5*	3	ND

[a] Mean values (n = 3). The values in italics are significantly lower or higher than the control value at $P \leq 0.05$
ND: not determined
U, A: unmodified and acetylated RPI, respectively

that is an important factor affecting organoleptic quality and microbiological stability of margarines (ZSCHALER, 1977). Incorporation of 0.25% of RPI to margarine model system additionally decreased intensity of fat spattering under frying, thus frying weight losses were also decreased (Table 5). However, RPI tested here showed poor properties in comparison to lecithin. In the margarine model systems the acetylated RPI has not been found superior to the unmodified RPI.

3. Conclusion

The results presented herein have proved that acetylation applied during extraction and isolation of rapeseed protein is an effective method for protein modification, as well as to increase the yield of the protein isolation process. Among examined rapeseed cultivars, improved rapeseed (LE, DL) showed better usefulness since a higher yield of protein isolates was obtained. The acetylated RPI contained considerably reduced to negligible amount of phytic P and improved functional properties. Positive effect of acetylation, especially on improvement of nitrogen solubility, water absorption capacity and emulsifying properties creates new possibilities of rapeseed utilization. However, the favourable functional properties of the acetylated RPI that have been shown by simple tests were not proved in the mayonnaise and margarine model systems. Improvement of emulsion quality and differences among tested isolates were not enough significant to consider acetylated RPI as a functional additive in the examined food model systems. Further study on the acetylation process and improvement of functional properties in respect to a certain food system still remains to be undertaken.

Literature

BOOTH, V. H. (1971): Problems in the determination of FDNB-available lysine. *J. Sci. Fd Agric., 22,* 658–664.

CHO, Y. S. & THOMPSON, L. U. (1984): Preparation behavior of nitrogen, phytic acids and minerals in rapeseed modified by acylating agents. *J. Fd Sci., 49,* 765–770.

CHOI, Y. R., LUSAS, E. W. & RHEE, K. C. (1983): Molecular structure and functionalities of protein isolates prepared from defatted cottonseed flour succinylated at various levels. *J. Fd Sci., 48,* 1275–1279.

EL NOCKRASHY, A. S., MUKHERJEE, K. D. & MANGOLD, H. K. (1977): Rapeseed protein isolates by countercurrent extraction and isoelectric precipitation. *J. agric. Fd Chem., 25,* 193–197.

FEENEY, R. E., YAMASAKI, R. B. & GEOGHEGAN, K. F. (1982): Chemical modification of proteins: an overview. *Adv. Chem. Series, 198,* 3–55.

FRANZEN, K. L. & KINSELLA, J. E. (1976a): Functional properties of succinylated and acetylated soy protein. *J. agric. Fd Chem., 24,* 788–795.

FRANZEN, K. L. & KINSELLA, J. E. (1976b): Functional properties of succinylated and acetylated leaf protein. *J. agric. Fd Chem., 24,* 914–919.

GWIAZDA, S. & KOCON, J. (1979): The influence of drying method on microstructure, physicochemical and sensoric properties of pea protein concentrates. *Acta Alimentaria Polonica, 5,* 71–80.

NAKAI, S. (1983): Structure-function relationships of food proteins with emphasis on the importance of protein hydrophobicity. *J. agric. Fd Chem., 31,* 672–677.

OBERLEAS, D. (1971): Determination of phytate and inositol phosphates. *Methods Biochem. Anal., 20,* 87–101.

RUTKOWSKI, A. & GWIAZDA, S. (1986): Some specific properties of rapeseed protein. Paper presented at 18th ISF Congress, Münster, FRG.

THOMPSON, L. U. & RENIERS, D. (1982): Succinylated whey protein concentrates in coffee whitener and salad dressings. *J. Dairy Sci., 65,* 1135—1139.

THOMPSON, L. U. & CHO, Y. R. (1984a): Effect of acetylation upon extractability of nitrogen, phytic acid and minerals in flour and protein concentrates. *J. Fd Sci. 49,* 771–776.

THOMPSON, L. U. & CHO, Y. R. (1984b): Chemical composition and functional properties of acylated low phytate rapeseed protein isolate. *J. Fd Sci., 49,* 1584–1587.

THOMPSON, L. U., REYES, E. & JONES, J. W. (1982a): Modification of the sodium hexametaphosphate extraction-precipitation technique of rapeseed protein concentrate preparation. *J. Fd Sci., 47,* 982–988.

THOMPSON, L. U., RENIERS, D. & BAKER, L. (1982b): Succinylated whey protein concentrates in wieners and meat patties. *J. Dairy Sci., 65,* 1715–1720.

THOMPSON, L. U., RENIERS, D., BAKER, L. & SIU, M. (1983): Succinylated whey protein concentrates in ice cream and instant pudding. *J. Dairy Sci., 66,* 1630–1634.

ZSCHALER, R. (1977): Methode zur Bestimmung der Wassertröpchenverteilung in Wasser-in-Öl-Emulsionen. *Fette Seifen Anstr. Mittel., 79,* 107–111.

Acta Alimentaria, Vol. 18 (4), pp. 361–385 (1989)

S-METABOLISM OF METHIONINE-RICH YEASTS

A. HALÁSZ, B. MÁTRAI and ALI MUAYAD

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary

(Received: 30 March 1988, accepted: 27 September 1988)

Mutants were produced by mild mutagenic agents such as UV-radiation and nitrite treatment, from yeast strains belonging to different genera (Candida, Rhodotorula and Saccharomyces) *Candida guilliermondii* CBS 5256, *Candida utilis* CBS 5609, *Rhodotorula glutinis* CBS 315, *Saccharomyces carlsbergensis*. The primary selection has been based on higher sulfate requirement assumed. The diameters of the colonies inoculated by replica plating technique and grown on media rich/deficient in sulfate were compared and those of increased sulfate requirement were separated. About 25% of these showed an increased methionine concentration.

Norleucine has been used as methionine antagonist and the resistance of the mutants was compared to that of the parent strains. Our assays showed that the mutants selected were more sensitive against norleucine as compared to the untreated strains. Thus, the higher methionine content cannot be attributed to homoserine-O-transsuccinase derepression.

Simultaneously to the increased sulfate requirement, the mutants responded to a higher methyl donor concentration also by an enhanced growth.

In methionine enriched mutants also the lipoic acid concentration of the yeast increased in parallel to the augmentation of sulfurous amino acid concentration. The concentrations of both components react very sensitively to the aeration intensity applied during fermentation and these begin to fall at the value of 200 mmol $O_2 h^{-1} l^{-1}$.

This phenomenon can be explained by the utilization of the sulfur derived from the sulfate, which is in several reduction steps, converted to sulphide by the yeast. In this process, lipoic acid plays an active role as the prosthetic group of sulfate reductase.

Sulfur present in sulfate form has been found to be a much better sulfur source than methionine. Applying this latter, a slower cell growth can be observed due to sulfur limitation.

Our experimental results indicate that with the mutants produced, the sulfate reduction step became more efficient and provided an increased methionine content.

Keywords: yeast, s-metabolism, methionine enrichment

The deficient methionine and triptophane provision can be taken almost as a people's disease and this, too, contributes to the frequent incidence of various pathological processes (hepatic disease, anaemia). The daily methionine requirement of an adult is about 2.8 g, and this is satisfied only to about 63%, even in case of adequate protein consumption.

In the feeding of monogastric animals, methionine is also an essential amino acid, therefore the amino acid deficiency is overcome by supplementing feeding diets with methionine doses. However, according to feeding trials, the free amino acid shows a more rapid resorption and thus, only 30–40% of

the added methionine is utilized (GEBHARDT et al., 1977; BURACZEWSKA et al. 1977).

Thus the production of a biomass with increased methionine content, constitutes a more effective means for supplementing methionine deficient feed.

KOMATSU and co-workers (1974) successfully produced a mutant rich in methionine from *Candida petrophilum* ATCC 20226. The methionine content of the mutant was about 40% higher as compared to the original strain. OKANASHI and GREGORY (1970) produced, from *Candida tropicalis*, a mutant with a methionine concentration higher by 41%. The increase in methionine concentration was practically entirely the result of the rise of free methionine concentration. KOMATSU and co-workers (1974) reported that during active cell growth, in a shaken culture, the pool methionine concentration was nearly unchanged but decreased in the declining phase of proliferation.

It is worth giving consideration to the proper selection of the parent strain for mutant production as the methionine content of yeasts varies between wide limits, 0.17–1.0% (CHIAO & PETERSON, 1953).

ANDERSON and JACKSON (1957) reported the same for bacteria. Among yeasts, mainly the genus Rhodotorula was found rich in limiting amino acids.

The key-enzyme of methionine synthesis is homoserine-O-transacylase. This is repeatedly de-repressed in feedback resistant mutants. The enzyme is resistant against methionine, S-methyl-methionine, ethionine and norleucine and it is less sensitive to S-adenosyl-methionine than the wild strain LAWRENCE et al. 1968).

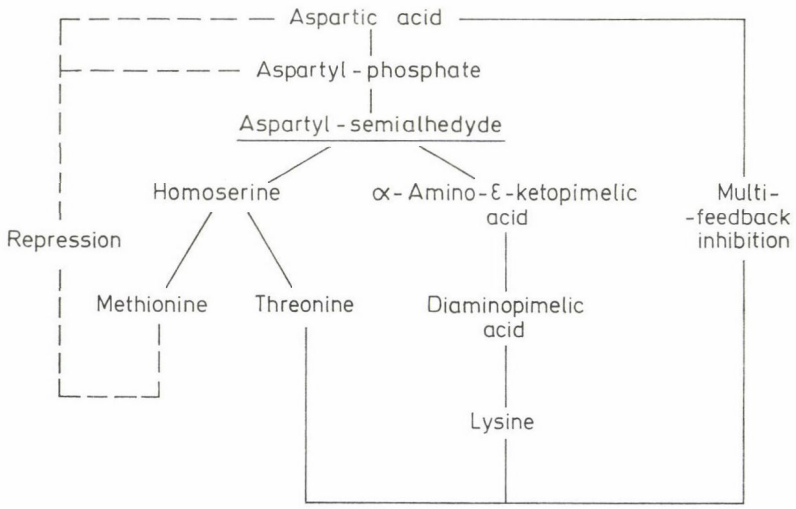


Fig. 1. Ramified biosynthetic pathway of methionine, threonine and lysine formation

In feedback resistant yeast mutants, pool methionine concentration increases significantly and in numerous cases, the amount of methionine excreted into the surroundings increases as well. In strains defective to S-adenosyl-methionine synthesis, the enrichment of pool methionine can be observed too, the formed compound being the metabolite of methionine.

The precursor of methionine formation is aspartic acid semi-aldehyde, similar to the biological synthesis of threonine, lysine and isoleucine (Fig. 1).

The first specific precursor of methionine is O-succinyl-homoserine formed through homoserine acylation catalyzed by homoserine-O-trans-succinase (SMITH, 1975). Feedback inhibition is exerted on the enzyme by S-adenosyl-methionine and S-methyl-methionine.

The mutants resistant to ethionine, S-methyl-methionine and norleucine are able to produce methionine in excess and are, therefore well utilizable for mutant selection.

GREENE and co-workers (1970) found accumulation of free methionine in the case of low S-adenosyl-methionine synthetase level.

The selection of mutants producing the desired product in greater quantities is a well-proved method in strain improvement work. Another requirement towards the mutants to be considered in our work is that the cells should not excrete the excess amino acid into the culture medium.

UV-irradiation is often applied for the production of mutation though its specific effect is little-known. We have more knowledge about the action mechanism of nitrite-treatment but there is no possibility to control the site of "defect" in either of the methods.

Therefore, the cells most favourable for our trials have to be separated from the variety of possible changes with thorough considerations.

In our actual work, manipulation carried out with yeast strains of several genera is reported. As a result, we succeeded in producing new mutants rich in methionine and the role of fermentation parameters could be evaluated as well.

Materials and methods

1.1. Yeast strains investigated

For the production of yeast mutants rich in methionine, a new yeast strain belonging to a different genus has been studied. Further, the stability of the mutants B_1 of *Candida guilliermondii* was investigated and the genetical stability of the methionine-rich mutant obtained from *Rhodotorula glutinis* was studied (Table 1).

Table 1

Yeast strains investigated

Name of the yeast	RNA (%)	Protein (%)	Methionine (% on dry matter basis)
Rhodotorula glutinis CBS 315	—	47.7	0.8
Candida guilliermondii CBS 5256	8.0	46.5	0.5
Candida utilis CBS 5609	6.0	41.1	0.65
Saccharomyces carlsbergensis	7.0	47.1	0.5

1.2. Culture media used

In our work, liquid and solid malt media and liquid glucose media — rich/deficient in sulfate — were used.

For the isolation of the mutants and for the production of yeast mutants needed for chemical analyses, yeast was grown in Petri dishes on a solid enrichment (II) culture medium.

Composition of the individual culture media:

I. Culture medium, deficient in sulfate

Urea	1.93 g dm^{-3}
$MgSO_4 \cdot 7H_2O$	2.5 mg dm^{-3}
KH_2PO_4	0.14 g dm^{-3}
$Na_2HPO_4 \cdot 12H_2O$	0.8 g dm^{-3}
NaCl	1.0 g dm^{-3}
glucose	2.0%

II. Sulfate-rich culture medium

$MgSO_4 \cdot 7H_2O$	0.1 g dm^{-3}
KH_2PO_4	0.14 g dm^{-3}
$NaHPO_4 \cdot 12H_2O$	1.0 g dm^{-3}
$(NH_4)_2SO_4$	4.0 g dm^{-3}
yeast autolysate (10%)	10.0 cm^3 dm^{-3}
glucose	2.0%

III. Culture medium

The composition of the culture medium is similar to (II), there is a difference only in the carbon source: 1% glucose + 1% maltose.

All culture media were stabilized with 2% of previously washed and dried agar-agar.

IV. Solid culture medium for growing Saccharomyces carlsbergensis

Malt	3.25 g dm^{-3}
yeast autolysate	100.00 cm^3 dm^{-3}
peptone	5.00 g dm^{-3}
glucose	10.00 g dm^{-3}
agar-agar	20.00 g dm^{-3}

V. Synthetic liquid culture medium for the propagation of yeasts in shaken culture

Similar to (II), but contains no yeast autolysate.

Malt-containing culture medium

Seventy g malt are weighed and added to 1 dm^3 water, then autoclaved up to 0.5 bar. After filtering, the refractive index is adjusted to 5 by a dilution with distilled water. It is again autoclaved up to 0.516 bar. For obtaining a solid medium, the pH has to be adjusted to about 5.5–6 and 2% agar-agar is added.

1.3. Determination of protein content

The protein content of the biomass has been determined by the Kjeldahl method with the aid of the automatic apparatus Kjelfoss.

1.4. Determination of amino acid composition

For our selection work, the methionine content of yeast has been determined from hydrochloric acid hydrolyzate by a microbiological method according to BARTON-WRIGHT (1977) using the *Leuconostoc mesenteroides* P_{60} auxotroph deficiency mutant as test microorganism. Complete amino acid composition was determined in the automatic amino acid analyzer type Aminochrom (Labor MIM, Hungary) from a sample hydrolyzed for 20 h with 6 mol l^{-1} HCl.

1.5. Hydrolysis of the yeast

Fifty mg dried and powdered yeast was weighted into a vial, 20 cm^3 6 N HCl added, the vial sealed and held at 105 $\pm$ 2 °C for 24 h. The vial was opened after cooling to room temperature, the hydrolysate transferred without residue into an evaporating dish and HCl was evaporated in vacuo with several washings. The residue was transferred into a plugged test tube with 3 cm^3 distilled water.

1.6. Determination of nucleic acid content (RNA)

The dried yeast was hydrolyzed with 1 mol l^{-1} $HClO_4$ in a water bath of 100 °C for 20 min. The cooled sample was centrifuged and the absorbance of the supernatant measured at 270 nm in a 1 cm quartz cell against 1 mol l^{-1} $HClO_4$.

1.7. Method for the production of yeast mutants

The production of yeast mutants was tried with two kinds of mutagenic agents, with nitrite, and with UV-irradiation, respectively. Genetic manipulation was performed according to BOWIEN and SCHLEGEL (1972) applying nitrite concentration and treating time established by us.

UV-irradiation was carried out with a germicide lamp (Tungsram, Budapest).

The pre-treatment of yeast cells was similar to that mentioned above, the irradiation time being selected according to the destruction curve measured. For irradiation, 5 cm^3 of suspension was weighted into a Petri dish and placed at a 15 cm distance from the lamp.

1.8. Methods applied for the selection of methionine-rich mutants

1.8.1. Separation of methionine-rich mutants according to increased SO_4^{2-} requirement. Following mutagenic treatment, a 72-h incubation in a shaken culture in culture medium (II) was applied for the development of mutation and the enrichment of mutants. For the separation of methionine-rich mutants, the colonies spread on malt culture medium (I) were transferred to sulfate-deficient (I) and sulfate-rich (II) synthetic glucose culture media by replica plating technique. The colonies being of greater diameter on sulfate-rich culture medium than in sulfate-deficient conditions were inoculated on to sulfate-rich agar slants. Comparisons were drawn between the methionine content of these mutants in relation to the parent strain.

1.8.2. Selection of mutants using an amino acid antagonist. The mutant rich in metabolite shows a better tolerance against higher concentrations of anti-metabolites and this phenomenon is often used for selection purposes (SCHERR & RAFELSON, 1962).

In our assays, norleucine was added to culture medium (II) and the beginning and extent of growth were studied in a shaken culture, in comparison to the control without norleucine. For the selection of the mutant, the inhibiting concentration determined for the parent strain was used as indicator.

1.8.3. Mutant selection using a methionine-homologous methyl donor. Another special requirement of methionine synthesis is — beside sulfate — the methyl donor (Fig. 2.), therefore it can be assumed that also the methyl

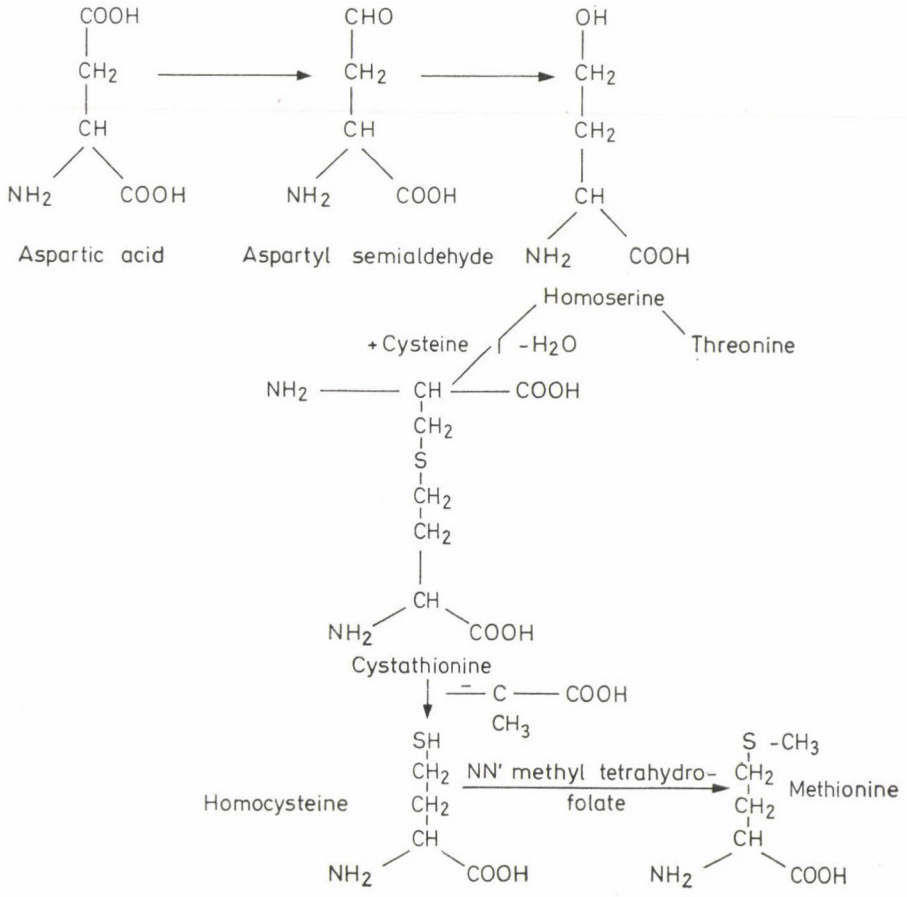


Fig. 2. Role of methyl donor in methionine biosynthesis

donor requirement of methionine-rich mutant should be higher than that of the original strain.

To our selections, S-methyl methionine sulfonium chloride (vitamin U) was applied in a concentration of 10–60 $\mu g\ cm^{-3}$ to culture medium (II) and the colony sizes were studied in comparison to those grown on plain culture medium (II).

The strains studied were inoculated with sterile wooden rodlets on to Petri dishes, to a place previously marked. After the development of colonies, they were transferred with sterile velvet on various culture media with vitamin U content.

1.9. The yeast propagation methods applied

1.9.1. In Petri dishes. The yeast streaked on the culture medium stabilized with 2% washed agar was propagated at 32 °C.

1.9.2. In shaken culture. Infusion flasks of 500 cm^3, containing 150 cm^3 culture medium were applied for shaken culture. The inoculation was carried out with yeast grown in Petri dishes to 1% yeast concentration. The propagation was carried out in a shaker adjusted to 32 °C.

1.9.3. In tube fermentor. A double-wall glass tube of 6 cm inner diameter was used as fermentor. To its bottom opening a gas inlet tube was attached through sintered glass filter G-4. The propagation temperature required (32 °C) was assured by the circulation of water of adequate temperature.

The propagations were carried out at aeration intensities of 250, 500, 750, 1000 and 1250 l h^{-1}, equivalent to 28, 44, 51, 71 and 84 mmol O_2 $l^{-1}h^{-1}$ oxygen transfer rates, respectively.

The pH of the culture medium was held at a constant value by addition of 2.5% NH_4OH solution.

1.9.4. In laboratory fermentor. To our trials, two kinds of laboratory fermentors were applied: types Biofer (Hungary) and Chemap (Switzerland). With the changing of stirrer revolutions and the rate of air flow, oxygen transfer rates of 60–200 mmol O_2 $l^{-1}h^{-1}$ could be established.

During propagation, pH and temperature were controlled automatically.

1.10. Determination of oxygen transfer rates

The oxygen transfer rates of various propagating devices were determined according to COOPER and MILLER (1944).

1.11. Determination of lipoic acid content of the yeast

The lipoic acid content of the yeast was determined according to MUAYAD and co-workers (1983).

1.12. Investigation of sulfur uptake of brewer's yeast in labelled culture medium

For the trials, sodium sulfate S^{35} and methionine S^{35} were used as sulfur sources.

The liquid culture medium was free from yeast extract and sulfate, and was supplemented with a standard biotin solution (60 μg dm^{-3}).

The nutrient liquor of 2% glucose content was inoculated with 0.5% inoculum. As sulfur source $Na_2{}^{35}SO_4$ at 1 mCi (37MBq) and S^{35} methionine, respectively, were used.

Following the inoculation with yeast sodium sulfate was directly added to the sterile culture medium, then this was agitated for 24 h at 37 °C with a magnetic stirrer. The yeast suspension was centrifuged (4000 min^{-1}) for 10 min. The precipitate was suspended in 20 cm^3 water and recentrifuged. This procedure was twice repeated using 20 cm^3 ethyl alcohol for the last washing.

The yeast was transferred from the centrifuge tube to a 50 cm^3 flask with alcohol and evaporated to dryness in vacuo. Supernatants obtained by repeated centrifugation were combined, diluted with water to 100 cm^3 and activity was measured by the liquid scintillation method. Hydrolysis was carried out with 25 cm^3 6 mol l^{-1} HCl, in an oil bath of 100–110 °C, for 5 h.

The dark brown hydrolysate was clarified with charcoal, washed with water and the clear yellow solution was evaporated in vacuo for the removal of excess HCl. The residue was diluted to 100 cm^3 with water and the activity measured with the liquid scintillation system.

The determination of the methionine content of the protein was carried out with radio-TLC method. After dilution to 100 cm^3 with water, the hydrolysate was evaporated in vacuo to dryness and the residue dissolved in 3 cm^3 water. Ten μl of this and along with it — as a standard — methionine S^{35} were applied to Polygram Ionex-25 SA-Na ionexchange thin layer and run in sodium citrate (0.25 mol l^{-1} citric + 0.25 mol l^{-1} sodium hydroxide, pH 3.3) solution. After drying, a radio chromatogram was made from the layer, in a Berthold LB-2721 instrument.

The part containing methionine was separately scraped from the layer, then the other parts of the layer were scraped together, eluted and their activities measured by the liquid scintillation method.

The results of the two measurements were taken as 100% and from this, the percentage of methionine was calculated.

2. Results

2.1. Production of methionine-rich mutants with Rhodotorula glutinis *CBS 315*

2.1.1. Generation of mutation by nitrite treatment. In order to obtain an effective mutation, the influences of nitrite concentration and treatment time on cell destruction were studied. The dependence on nitrite concentration was observed with 1.7, 3.4, 5.1, 6.8, 8.5 and 10.2 mg cm^{-3} $NaNO_2$ concentrations and 30 min treatment time. The viable germ count was determined by the plate pouring method. The effect of nitrite concentration is shown in Table 2.

The time dependence of the treatment was determined for the concentration of 3.4 mg cm^{-3} $NaNO_2$.

Based on our findings, a treatment time of 30 min and concentrations of 3.4, 6.8 and 8.5 mg cm^{-3} $NaNO_2$ were applied producing the mutation.

Following enrichment, the colonies streaked on malt culture medium were inoculated by replica plating on culture media (I) and (II). The cells separated according to colony diameters were analysed for methionine content. From the colonies separated from the first selection, only six proved to be viable after reinoculation to culture medium (II).

From the well-growing colonies, the quantity needed for methionine

Table 2

Cell destruction of Rhodotorula glutinis *CBS 315 as an effect of* $NaNO_2$

$NaNO_2$ (mg cm^{-3})	$\lg N_0/N$
1.7	1
3.4	1
6.8	1
8.5	2
10.2	2

N_0: living cell number before treatment
N: living cell number after treatment
Treatment time: 30 min

and protein determinations was produced in shaken cultures. Based on methionine values determined by the microbial method, only one mutant was found richer in methionine (Table 3).

2.1.2. Generation of mutation by UV-irradiation. In preliminary trials, it was found that a 60-min UV-treatment of strain CBS 315 reduced the viable germ count by two orders and therefore this was used for genetical manipulation.

From the colonies grown on culture media (I) and (II), only two colonies could be selected on the basis of colony diameters. Based on methionine and protein values determined from the cell material grown in shaken culture, the strain 315 UV_1 could be taken as a mutant richer in methionine (Table 4).

2.2. Production of methionine-rich mutants with Saccharomyces carlsbergensis *(brewer's yeast)*

2.2.1. Generation of mutation by nitrite treatment. For *Saccharomyces carlsbergensis* the nitrite sensitivity of the strain was determined as well.

Table 3

Methionine and protein concentrations of the mutants obtained by NO_2 *treatment from* Rhodotorula glutinis *CBS 315*

Sign of sample	Methionine[a] (% related to solids)	Protein[b]
CBS 315	0.80	44.7
315/1	0.65	—
315/2	0.50	—
315/3	0.75	42.7
315/4	0.50	40.3
315/5	*0.90*	38.8
315/6	0.80	41.1

Values are the mean of three replicates
[a] standard deviation of the method ±10%
[b] standard deviation of the method ±5%

Table 4

Methionine and protein contents of the UV mutants of Rhodotorula glutinis *CBS 315*

Sign of sample	Methionine[a] (% related to solids)	Protein[b] (% related to solids)
CBS 315	0.80	44.7
315 UV_1	1.00	40.6
315 UV_2	0.50	39.8

Values are the mean of three replicates
[a] standard deviation of the method: ±10%
[b] standard deviation of the method: ±5%

The changes in viable germ count found with 10 and 30 min treatment times and 3.4, 6.8 and 10.2 mg cm^{-3} $NaNO_2$ concentrations are summarized in Table 5.

Mutant production was performed with 3.4 and 10.2 mg cm^{-3} $NaNO_2$ concentrations and 30 min treatment time. Separating the colonies indicating a higher sulfate requirement as based on the methionine determination it has been found that the four colony forming units all showed a reduction in methionine, protein and RNA concentrations as related to solids (Table 6).

Table 5

Cell destruction of Saccharomyces carlsbergensis *as an effect of $NaNO_2$ treatment* (30 min)

$NaNO_2$ concentration (mg cm^{-3})	$\lg N_0/N$
3.4	2
6.8	2–3
10.2	3

Table 6

Methionine, protein and RNA contents of S. carlsbergensis *mutants obtained by NO_2 treatment*

Sign of sample	Methionine[a] (% related to solids)	Protein[b] (% related to solids)	RNA[b]
S. carlsbergensis	0.5	47.1	7.0
S_{34}	0.3	32.7	4.6
SC_{34}	0.4	31.8	4.0
SC_{1021}	0.25	32.2	5.8
SC_{1022}	0.25	31.7	4.0

Values are the mean of three replicates
[a] standard deviation of the method: ±10%
[b] standard deviation of the method: ±5%

Table 7

Methionine, protein and RNA contents of UV mutants of S. carlsbergensis

Sign of sample	Methionine[a]	Protein[b]	RNA[b]
	(% related to solids)		
S. carlsbergensis	0.5	47.1	7.0
SC_1	*0.65*	38.6	7.0
S_2	0.32	42.4	6.2
S_3	0.50	52.8	9.8
S_4	*0.80*	54.8	11.6
S_5	0.50	49.2	9.7
S_6	*0.68*	33.6	4.2
S_7	0.25	36.0	5.8
S_8	0.48	54.0	8.0
S_9	0.43	46.0	8.6
S_{10}	*0.75*	62.2	7.6

Values are the mean of three replicates
[a] standard deviation of the method: $\pm 10\%$
[b] standard deviation of the method: $\pm 5\%$

2.2.2. Generation of the mutation with UV-irradiation. For *S. carlsbergensis* already a 30 min UV-treatment resulted in a viable germ count reduction of 2 orders, thus this treatment time was chosen for the production of the mutant.

Based on increased sulfate requirement, 15 colonies could be selected but from them only 10 proved to be viable after reinoculation. The viable cells were grown on culture medium (II) in a shaken culture, then the methionine content was determined. Results are summarized in Table 7.

Based on methionine values, four mutants were richer in methionine than the initial brewer's yeast. The methionine concentration related to protein was highest in mutant S_6.

2.3. Production of methionine-rich mutants with Candida utilis *CBS 5609*

Taking into consideration the nitrite sensitivity of the strain *Candida utilis* CBS 5609, 6.8 and 8.5 mg cm^{-3} $NaNO_2$, resp., and 30 min treatment time were used to obtain a viable germ count reduction by 2 orders (Table 8). Seven colonies could be separated based on increased sulfate requirement. The methionine, protein and RNA contents of the yeast grown in shaken culture are given in Table 8.

The results indicate that the mutants FT_3 and FT_4 are richer in methionine, with a satisfactory protein content.

Table 8

Methionine, protein and RNA contents of NO_2 mutants of Candida utilis *CBS 5609*

Sign of sample	Methionine[a]	Protein[b]	RNA[b]
	(% related to solids)		
C. utilis SBS 5609	0.65	41.1	6.0
FT_3	*0.77*	42.6	7.0
FT_4	*0.75*	42.4	7.0
FT_5	0.38	31.6	4.0
FT_6	0.40	43.8	6.0
FT_7	0.50	43.8	6.0
FT_8	0.50	39.1	6.1
FT_9	0.55	33.8	5.0

Values are the mean of three replicates
[a] standard deviation of the method: ±10%
[b] standard deviation of the method: ±5%

2.4. *Production of methionine-rich mutants with the strain CBS 5256 of* Candida guilliermondii

2.4.1. Generation of the mutation by $NaNO_2$ treatment. For the *Candida guilliermondii* strain 5256, of 3.4, 6.8 and 10.2 mg cm^{-3} concentrations resulted in D_{10} values of 30, 20 and 10 min, respectively. In order to arrive to a good mutation effect, the conditions of the treatment were chosen to result in a 99–99.9% destruction in the 10^9 cell per cm^3 yeast suspension (60 min, 6.8 mg cm^{-3} $NaNO_2$).

From the methionine-rich mutants, selected after simple mutation double mutants were produced by repeating. The methionine-rich individuals were selected from the latter. The methionine, protein and RNA contents determined in the yeast mass grown on culture medium (II) are presented in Table 9.

Table 9

Methionine, protein and RNA contents of the mutants obtained by NO_2 treatment from Candida guilliermondii *CBS 5256*

Sign of sample	Methionine[a]	Protein[b]	RNA[b]
	(% related to solids)		
C. guilliermondii CBS 5256	0.50	47.2	8.0
1× mutated 1	0.65	47.2	13.4
1×mutated 2	0.70	44.5	7.6
2×mutated from B_1	0.80	45.7	10.0
2×mutated from B_2	0.90	47.5	9.9
2×mutated from C_4	0.75	50.4	9.6
2×mutated from D_2	0.65	42.1	9.6

Values are the mean of three replicates
[a] standard deviation of the method: ±10%
[b] standard deviation of the method: ±5%

2.4.2. Generation of the mutation with UV-irradiation. With *Candida guilliermondii* CBS 5256, a 60-min UV irradiation caused only a 90% cell destruction. From the mutants of increased sulfate requirement none proved to be enriched in methionine.

2.5. Production of methionine-rich mutants with Candida guilliermondii *CBS 812*

2.5.1. Generation of the mutation by $NaNO_2$ treatment. The *Candida guilliermondii* strain CBS 812 proved to be very sensitive to $NaNO_2$, its D_{10} value (3.4 mg cm^{-3}) was 15 min. From the 1400 colonies grown after the mutagenic treatment none showed an increased growth on sulfate-rich culture medium.

2.5.2. Generation of the mutation with UV-irradiation. The strain CBS 812 seemed to be very sensitive to UV-irradiation.

Its D_{10} value was 2.18 min. Based on an increased sulfate demand, 26 colonies could be separated out of 2080. After the determination of methionine concentration, 4 colonies proved to be enriched in methionine (Table 10).

Table 10

Methionine, protein and RNA contents of UV-mutants of Candida guilliermondii *CBS 812* (propagation in Petri dish)

Sign of sample	Methionine[a]	Protein[b]	RNA[b]
	(% related to solids)		
Candida guilliermondii			
CBS 812	0.50	44.3	10.8
120 s	0.83	41.2	6.8
180 s	0.80	52.0	5.6
240 s	0.85	43.0	6.3
300 s	0.88	39.0	7.3

Values are the mean of three replicates
[a] standard deviation of the method: ±10%
[b] standard deviation of the method: ±5%

Table 11

Methionine, protein and RNA contents of 3x UV-mutants of Candida guilliermondii *CBS 812* (propagation in tube fermentor)

Sign of sample	Methionine[a]	Protein[b]	RNA[b]
	(% related to solids)		
CBS 812	0.53	43.9	9.2
S/4	0.71	44.5	6.2
11	0.85	45.1	5.7

[a] standard deviation of the method: ±10%
[b] standard deviation of the method: ±5%

A triple mutation with UV-irradiation resulted in stable mutants of *Candida guilliermondii* CBS 812. The methionine, protein and nucleic acid contents of the yeast mass grown in tube fermentor are presented in Table 11.

2.6. Trials aimed at improving the efficiency of methionine-rich mutant selection

As a first step, increased sulfate requirement was utilized for the separation of methionine-rich mutants from the cells of the parent strain and from the mutants changed in other directions, respectively. The sulfur required for methionine synthesis is taken up by the yeast cells from the sulfate content of the culture medium and utilized, reduced in several steps to sulfite, then to sulfide, for homocysteine synthesis (Fig. 3.). Methionine is then formed by methylization of homocysteine.

Taking the increased sulfur utilization as a base, the growth of colonies streaked after mutation was investigated in sulfate-deficient and in sulfate--rich culture media. Comparing the diameters of the colonies obtained by replica plating, the cells assumed to be methionine-rich, were separated.

This selection method proved to be successful. Nevertheless, we were not satisfied by the efficiency of the selection method, as after methionine determination only about 25% of the assumedly favourable cells proved to be really

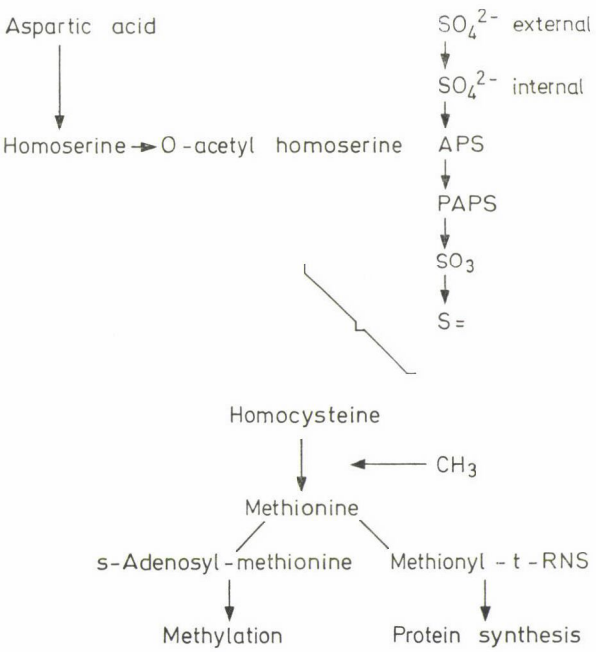


Fig. 3. Sulfur source side of methionine biosynthesis

methionine-rich mutants, and some showing negative changes for methionine were detected as well.

To improve our mutant separation work, two solutions were studied: utilization of a methionine antagonist and adding a methionine homologue.

2.6.1. Improvement of mutant selection efficiency by the use of norleucine. According to the literature it is a general experience that the methionine-rich mutant shows a better tolerance towards the antimetabolite and this phenomenon is often used in selection work (SCHERR & RAFELSON 1962).

From the methionine analogues, ethionine was used the most frequently as this proved to be suitable in the selection of methionine-rich mutants and in the stable maintenance (COLOMBANI et al., 1975; MORZYSKA et al., 1976).

LAWRENCE and co-workers (1968) observed a resistance against norleucine in methionine-rich mutants.

For the use of norleucine to selection the inhibiting concentration related to the parent strain has been determined in a first step.

For the selection of the concentration range, the inhibiting concentrations reported for ethionine were taken into consideration and thus first concentrations of 0.001–0.01% were applied in studying the effect of norleucine on *Candida guilliermondii* CBS 5256.

Shaken colonies were prepared for the assays and growth was measured after 24 and 48 h. Norleucine was added in concentrations of 0.001, 0.002, . . . 0.01%, as a total in 10 of concentrations. Based on the absorbance and pH changes of the colonies, no inhibiting effect was observed in the starting of growth, nor in its degree (Table 12).

The inhibiting norleucine concentration was investigated in the range of 0.01–1% for the strains CBS 5256 and CBS 315. Significant inhibition was

Table 12

Determination of the inhibiting norleucine concentration with C. guilliermondii *strain CBS 5256*

Norleucin concentration (%)	Absorbance of shaken culture						
	0 h	24 h			48 h		
		$\bar{x}_1$	±s		$\bar{x}_2$	±s	
0	0.64	0.50	0.05	10 times diluted	0.62	0.07	10 times diluted
0.001	0.64	0.58	0.07		0.65	0.06	
0.006	0.64	0.48	0.05		0.47	0.05	
0.009	0.64	0.59	0.06		0.51	0.05	
0.01	0.64	0.56	0.07		0.55	0.06	
0.05	0.20	0.10	0.03	without dilution	1.4	0.05	without dilution
0.1	0.20	0.80	0.05		1.4	0.08	
0.2	0.20	0.60	0.07		1.3	0.08	
0.5	0.20	0.40	0.05		1.4	0.10	
1.0	0.20	0.20	0.03		1.2	0.08	

$\bar{x}_1$, $\bar{x}_2$: mean values of three shaken culture samples
±s: standard deviation

observed only with 1% norleucine. The starting of growth was delayed for *Rhodotorula glutinis* CBS 315, while for *Candida guilliermondii* CBS 5256 the cell density was only one tenth of those formed at 0.01–0.05%, as this is presented in Table 12.

Norleucine sensitivity was compared for *Candida guilliermondii* strain CBS 5256 and mutant B_1. Based on values related to control growth, of the untreated cells a definitely slower growth could be observed for the parent strain and also for the mutant in the presence of 1% norleucine.

The mutant showed a higher sensitivity at this concentration than the parent strain and its inhibition was observed already at 0.5% norleucine (Table 13).

2.6.2. Improvement of the efficiency of mutant selection by use of a methionine homologue. Another special requirement of methionine synthesis beside sulfate is the methyl donor (Fig. 2), therefore it can be assumed, that the requirement of the methionine-rich mutant for this would be also higher than that of the initial strain.

S-methyl-methionine sulfonium chloride (vitamin U) is capable to play the role of methyl donor and to replace the methionine in this respect (McRorie et al., 1954; Hegedüs et al., 1977). In our experiments aimed at the improvement of selection, vitamin U was added to the sulfate-rich culture medium in concentrations of 10–60 μg cm^{-3} and the sizes of the colonies developed were compared to those produced on plain culture medium (II). As it can be seen in Fig. 4, the diameter of the colonies formed on the control culture medium was the smallest, and with the increase in added vitamin U concentration, the colonies gradually increased.

This could be observed for the original strains (CBS 5256 and CBS 315), as well as for the corresponding mutants (B_1 and CBS 315 UV_1). However, the stimulating on mutants was more expressed.

Table 13

Comparison of norleucine sensitivities of Candida guilliermondii *CBS 5256 and mutant B_1 in shaken culture*

Norleucine concentration	Absorbance of Culture CBS 5256			Culture B_1		
	Time (h)					
	0	24	48	0	24	48
0	0.2	0.84	1.4	0.2	0.28	0.59
0.5	0.2	0.44***	1.4	0.2	0.18***	0.19***
1.0	0.2	0.20***	1.2	0.2	0.12***	0.17***

Mean values of 3 parallel measurements
*** Very highly significant at $P \geq 99.9\%$ probability level

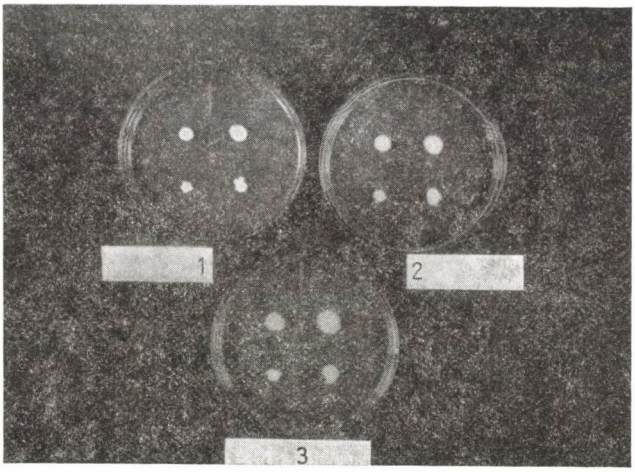


Fig. 4. Selection of methionine-rich yeasts on methyl-donor medium. 1: Control; 2: 20 μg Vitamin U; 3: 60 μg Vitamin U. Left upper colony 315 UV; right upper colony B_1; left lower colony CBS 315 UV_1; right lower colony CBS 5256

2.7. Utilization of the sulfur sources sulfate and methionine, respectively, by methionine-rich yeast

For our assays, methionine-rich *Saccharomyces carlsbergensis* propagated on malt wort, without aeration was used.

2.7.1. SO_4 utilization of S. carlsbergensis. $Na_2{}^{35}SO_4$ was used in a concentration corresponding to that used in culture medium (II). As a result of our preliminary trials, yeast extract was not applied because with this excess sulfate would have been introduced. This would have decreased the apparent incorporation percentage of the isotope sulfur derived from the S-labelled sodium sulfate. After a propagation of 48 h using $Na_2{}^{35}SO_4$ as sulfur source, 269.5 mg yeast obtained from 100 mg, the resulting plus was 169.5 mg. The remaining nutrient solution and the washing liquids gave a combined activity of 2 294 105 Bq, the activity incorporated in the yeast was 3.8, 87 106 Bq, thus the degree of sulfate incorporation was 94.43%.

The quantitative distribution of sulfur containing amino acids formed from the sulfate was determined, from the radio chromatogram of the hydrolysates obtained after hydrolysis of 2, 5, 10, 15 and 20 h. As it can be seen from Fig. 5, each hydrolysate showed three radioactive peaks, one of them for methionine and another for cysteine. The size of the nonidentified third peak decreased with increasing duration of hydrolysis. At the same time, the methionine content increased. The sum of activity percentages represented by the two peaks was constant.

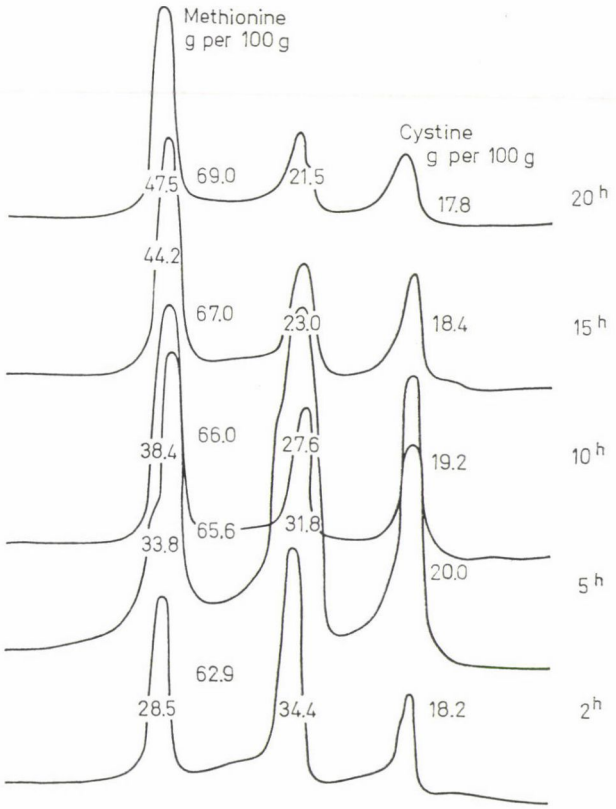


Fig. 5. Radiogram of the hydrolysis product of *S. carlsbergensis*. (Quantitative evaluation of the radiogram can be seen in Table 14)

Table 14

Investigation of ^{35}S incorporation in the hydrolysate of grown on $Na_2{}^{35}SO_4$ culture medium

Hydrolysis time (h)	Methionine (%)	Non-identified peak (%)	Cysteine (%)	Methionine + identified peak (%)
2	28.5	34.4	18.2	62.9
5	33.8	31.8	20.0	65.6
10	38.4	27.8	19.2	66.0
15	44.2	23.0	18.4	67.2
20	47.5	21.5	17.8	69.0

The non-identified peak can be assumed to be methionine-sulfoxide. This is in agreement with the changes with hydrolysis time in the amount of the substance to be identified and with its transformation to methionine, further with the R_f value of methionine-sulfoxide.

2.7.2. Methionine utilization of S. carlsbergensis. The culture medium used for the trials contained as sole sulfur sources S-labelled methionine in a quantity similar to the sulfate content of the sulfate culture medium. During propagation of 48 h, 206 mg yeast were formed from 100 mg i.e. the increase was, considerably less than the value observed for sulfate medium.

Based on the combined activity of nutrient liquid and washing solution, and on the labelled sulfur detectable in yeast, only 45% was incorporated from the methionine present in the culture medium. This incorporation ratio was lower than that found with the sulfate medium even when taking into consideration that the cell growth was less in the methionine containing medium. According to the radiochromatogram of the yeast hydrolysate, 45% of the uptaken activity was present in the form of methionine in yeast. This proves that the proportion of sulfur containing amino acids of *S. carlsbergensis* does not depend on the fact whether the sulfur source was sulfate of methionine.

2.8. The effect of oxygen transfer rate in yeast propagation on methionine and lipoic acid concentrations

In our work we found that the cell mass grown in Petri dishes showed the highest methionine content, for parent strains as well as for methionine-rich mutants. The methionine concentration of cells grown in shaken cultures or tube fermentor is considerably lower (Table 15).

Table 15

Methionine content of the mutant B_1 of Candida guilliermondii *CBS 5256 in cultures with different O_2 transfer rates*

Petri dish	1	Shaken culture	Column fermentor	Laboratory fermentors	
				Biofer	Chemap
		2	3	4	5
Methionine related to solids (%)	0.80 $\pm$0.07	0.70 $\pm$0.08	0.62 $\pm$0.06	0.62 $\pm$0.06	0.52 $\pm$0.05

Test of significance

	1	2	3	4
5	***	***	***	***
4	***	***	∅	
3	***	***		
2	***			

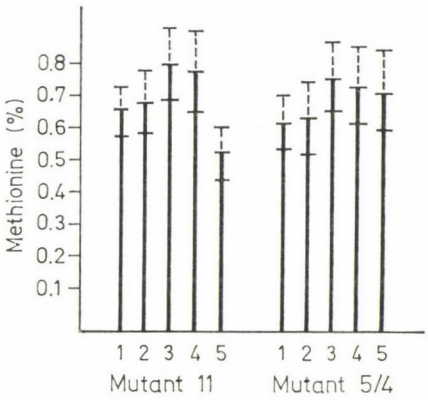
*** Very highly significant at $P \geq 99.9\%$ probability level
∅ no significant difference

Within the range of oxygen transfer rates of 28–84 mmol $O_2 l^{-1} h^{-1}$ up to 58 mmol $l^{-1} h^{-1}$ value methionine concentration was increased. However, with 84 mmol $l^{-1} h^{-1}$ it showed already a decrease as compared to the highest value measured for the mutants *Candida guilliermondii* 11 and 514, as it is to be seen in Figs. 6 and 7.

In laboratory fermentors provided with an agitator for increased oxygen supply, the methionine content of the biomass formed showed the results given in Fig. 8 for parent and mutant strains, with 70 and 220 mmol $O_2 l^{-1} h^{-1}$ oxygen transfer rates, respectively.

Methionine content was found to decrease with the intensification of the aeration.

With the increase in oxygen transfer rate the lipoic acid content of yeast cells underwent a change similar as shown by the summarizing data of Table 16.



	5	4	3	2
1	*	*	*	∅
2	*	*	*	
3	**	∅		
4	*			

11

	5	4	3	2
1	*	*	*	∅
2	*	*	*	
3	*	∅		
4	∅			

5/4

Fig. 6. Influence of oxygen transfer rate on methionine content of mutants 11 and 5/4 (propagation in tube fermentor). 1, 2, 3, 4, 5: oxygen transfer rates = 28, 44, 58, 71, 84 mmol O_2 $l^{-1} h^{-1}$. Methionine content % on dry material. * Significant at $P \geq 95\%$ probability level; ** highly significant at $P \geq 99\%$ probability level; ∅ no significant difference

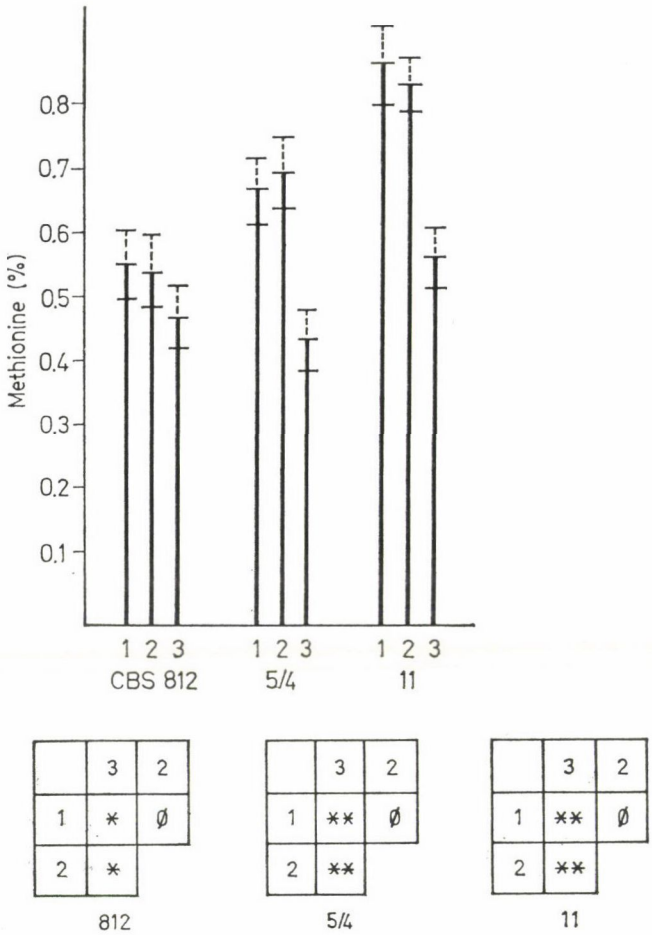


Fig. 7. Selection of methionine-rich yeasts on methyl donor medium. 1, 2, 3: oxygen transfer rates = 70, 150, 200 mmol O_2 $l^{-1}h^{-1}$. Methionine content % on dry material. * Significant at $P \geq 90\%$ probability level; ** highly significant at $P \geq 99\%$ probability level; ∅ no significant difference

3. Discussion

3.1. Production of methionine-rich mutants with gentle mutagenic agents

Our results prove that even gentle mutagenes can be effectively used for the production of methionine-rich yeast mutants, similarly to the powerful mutagenes applied.

With multiple mutation, the methionine content can be nearly doubled as compared to the initial value.

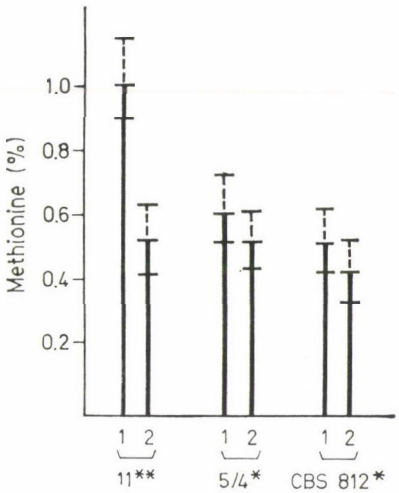


Fig. 8. Influence of oxygen transfer rate on the methionine content of *Candida guilliermondii* 812 and mutants 11 and 5/4 (propagation in laboratory fermentor type Chemap). 1, 2: oxygen transfer rate = 90, 200 mmol $O_2\,l^{-1}h^{-1}$. * Significant at $P \geq 95\%$ probability level; ** highly significant at $P \geq 99\%$ probability level

Table 16

Effect of oxygen transfer rate on the methionine nad lipoic acid contents of Candida guilliermondii *812 and mutant 11*

Strain	Transfer rates (O_2 mmol l^{-1} h^{-1})	Methionine (g per 100 g)		Lipoic acid (mg per 100 g)	
		$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
CBS 812	200	0.45	0.03	0.35	0.03
	50	0.60	0.04**	0.42	0.05*
11	200	0.75	0.08	0.35	0.04
	50	1.00	0.10**	0.47	0.05**

* Significant at $P \geq 95\%$ probability level
** Highly significant at $P \geq 99\%$ probability level

3.2. Mutant selection

The methionine-rich mutants produced with gentle mutagenes have an enhanced sulfate requirement and this can be used succesfully for their selection as well.

In contrast to the results of LAWRENCE and co-workers (1968) we found that the mutants are more sensitive to norleucine than the parent strains. This phenomenon indicates that in the methionine-rich mutants produced, the increase in the concentration of sulfur containing amino acids cannot be attributed to the derepression of homoserine-O-transsuccinase.

The increased methyl donor requirement, i.e. the adding of the methionine homologue vitamin U resulted in an increased colony diameter as compared to the control. This proves that the utilization in the formation of cell material, of the better sulfur supply in sulfate-rich media is accompanied by a higher methyl donor requirement. With the mutant of higher methionine content, this appears to a higher degree.

The results of investigation into the utilization of the sulfur source by methionine-rich yeast prove that the methionine-rich yeasts do not directly incorporate methionine. First this is oxidized to sulfate and then — though reductive pathways — to methionine.

Thus, growth conditions limited with respect to sulfur are created as compared to sulfate containing culture medium, resulting in a slighter cell multiplication.

The ratios of sulfur containing amino acids do not depend on the kind of sulfur source, i.e. sulfate or methionine but the absolute quantity for methionine containing medium is about 23% lower of sulfate containing medium as calculated from the percentages of cell mass and sulfur incorporation ($163 : 106 = 94.43 : x = 58$, in contrast to 45%, if the former corresponds to 100% methionine and the latter to 77%).

3.3. Effect of oxygen transfer rate on methionine and lipoic acid contents of yeast

Taking into consideration the values used in general for yeast propagation, the methionine and lipoic acid concentrations of the yeast, begin to decrease for the parent strains as well as for the mutants already at the low value of 200 mmol $O_2 l^{-1} h^{-1}$.

This phenomenon can be attributed to the utilization of sulfur originating from sulfate, that is transformed through several reductive steps to sulfide (HILTE et al. 1959). In this process, lipoic acid is the prostethic group in sulfate reductase involved in the transformation of SO_4 to SO_3.

4. Conclusions

The methionine-rich mutants produced with gentle mutagenic agents as nitrite and UV-irradiation have an increased sulfate and methyl donor requirement.

They show a higher sensitivity towards norleucine than the parent strain, thus the increased methionine concentration cannot be attributed to feedback resistance to the de-repression of homoserine-O-transsuccinase.

The increased lipoic acid content of the mutants and the sensitivity towards the oxygen supply of the media result in lower methionine and lipoic acid concentrations at higher O_2 transfer rates. This indicates that for the

mutants produced the sulfate reduction step became more effective and this results in an increased methionine content.

At the same time it is noteworthy that, in contrast to the mutants produced with powerful mutagenes, in our assays the increase of methionine concentration occurred not in the higher concentration of free amino acids but in methionine in peptide-linkage.

Literature

ANDERSON, R. & JACKSON, P. (1957): Essential amino acids in microbial proteins. *Appl. Microbiol., 6,* 369–373.

BARTON-WRIGHT, E. (1977): The microbial assay of the essential amino acids in compound feeding stuffs. *Analyst, 97,* 138–141.

BOWIEN, B. & SCHLEGEL, H. (1972): Isolierung und Characterisierung katabolischer Defektmutanten von *Hydrogenomonas eutrophastamm* H 16. *Arch. Mikrobiol., 87,* 203—219.

BURACZEWSKA, L., ZEDROWSKA, T. & BURACZEWSKI, S. (1977): The rate passage of synthetic lysine and dietary protein from the stomach to the intestine in pigs. *V. Int. Symp. on Amino Acids,* Budapest. Abstracts, p. 13.

CHIAO, J. & PETERSON, W. (1953): Yeasts' methionine and cystine contents. *Agric. Fd Chem., 1,* 1005–1006.

COLOMBANI, F., CHEREST, H. & ROBINSON-SZULMAISTER, H. (1975): Biochemical and regulatory effects of methionine analogues in *Saccharomyces cerevisiae. J. Bact., 122,* 375–384.

COOPER, C. M. & MILLER, S. (1944): Performance of agitated gas-liquid contactors. *Ind. Engng Chem., 36,* 504–507.

GEBHARDT, G., ZEBROWSKA, T. & KÖHLER, R. (1977): An attempt to estimate the endogenous nitrogen and true digestibility of protein and individual amino acids. *V. Int. Symp. on Amino Acids,* Budapest. Abstracts, p. 41.

GREENE, R., SU, C. & HOLLOWAY, C. (1970): S-adenosyl-methionine synthesis deficient mutants of *E. coli* K-12 with impaired control of methionine biosynthesis. *Biochim. biophys. Res. Commun., 38,* 1120–1126.

HEGEDÜS, M., BOKORI, J., WÖLLER, L., SÓS, E. & REMÉNYI, E. (1977): Determination of S-methyl-methionine sulfonium salts with a microbial assay and different chromatographic methods. *V. Int. Symp. on Amino Acids,* Budapest. Abstracts, p. 49.

HILTE, H., KITTLER, M. & KNAPPE, G. (1959): Die Reduktion von Sulfat in der Hefe. *Biochem. Z., 332,* 151–156.

KOMATSU, K., YAMADA, T. & KODAIRA, R. (1974): Isolation and characteristics of pool methionine-rich mutants of *Candida sp. J. Ferment. Technol., 52,* 93—99.

LAWRENCE, D., SMITH, D. & BOWBURY, R. (1968): Regulation of methionine synthesis in *S. typhimurium:* mutants resistant to inhibition by analogues of methionine. *Genetics, 58,* 473–492.

MCRORIE, R., GLAZNER, M., SKINNER, C. & SHIVE, W. (1954): Microbiological activity of the methylsulfonium derivative of methionine. *J. biol. Chem., 211,* 489–497.

MUAYAD, A., HALÁSZ, A., MÁTRAI, B. & SZALMA-PFEIFFER, I. (1983): Efficiency of different mutagenic treatments and protoplast fusion technique in producing methionine-rich mutants from yeasts. *Acta Alimentaria, 12,* 211—221.

MORZYSKA, E., SAWNOR-KORSZYNSKA, D., PASZENSKI, A. & GRABSKI, J. (1976): Methionine overproduction by *Saccharomycopsis* lipolytica. *Appl. environm. Microbiol., 32,* 125–130.

OKANISHI, M. & GREGORY, K. (1970): Isolation of mutants of *Candida tropicalis* with increased methionine content. *J. Microbiol., 16,* 1139–1144.

SCHERR, G. & RAFELSON, M. (1962): The direct isolation of mutants producing increased amounts of metabolites. *J. appl. Bact., 25,* 187–194.

SMITH, I. (1975): Sulfate transport in cultured tobacco cells. *Plant Physiol., 55,* 303–307.

Acta Alimentaria, Vol. 18 (4), pp. 387—394 (1989)

RESULTS OF A SENSORY COLLABORATIVE TEST OF SOME FOOD PRODUCTS

P. MOLNÁR

Institute of Food Control, H-1095 Budapest, Mester u. 81. Hungary

(Received: 18 April 1988; accepted: 15 January 1989)

The methodology of a collaborative sensory test, developed by the author, was described in detail in an earlier paper. This proved to be suitable for the determination of repeatability (r) and comaparability (R) of the sensory scoring system.

This paper gives an account of the results of eight collaborative tests utilizing the system developed. Bologna sausage as meat product, various kinds of beer, various kinds of cigarettes and confectionary products were evaluated by scoring. The r and R values obtained showed good agreement with those found in related literature and with those obtained by the author in collaborative tests carried out abroad and they can be utilized in the practice of quality control.

Keywords: sensory collaborative study, scoring, sensory test

On the basis of the methodology as developed and published earlier (MOLNÁR, 1989) the following products of the food industry were studied by collaborative scoring test: Bologna sausage, lager beer, various kinds of cigarettes and confectionary.

The collaborative tests were aimed at

— testing the collaborative testing system from many angles by applying it to different materials;

— determining the repeatability (r) and comparability (R) of the sensory scoring methods applied in the collaborative test;

— comparing the r and R values obtained in intralaboratory and interlaboratory sensory collaborative tests of the same products;

— checking the scoring methods by collaborative testing and suggesting improvements to their accuracy;

— controlling and developing the work of the participating panels.

1. Methods

The participating panels evaluated the products to be tested by methods specified in valid standards or drafts of standards or in lack of such standards by self-developed methods.

Results obtained in the course of the collaborative tests were processed and evaluated by the methodology as developed earlier (MOLNÁR, 1989).

2. Results

2.1. Sensory collaborative test of Bologna sausage

Of meat products Bologna sausage proved to be a highly suitable model for collaborative testing. Therefore it was used in several collaborative tests.

In the interlaboratory collaborative study two different evaluation methods were tested.

The first collaborative test was carried out on the basis of a draft of testing specification which was developed according to the well known scoring method with weighting factors and 20 total scores.

In the second collaborative test the so called "property descriptive method" (HUNGARIAN STANDARD, 1984) was applied according to which the scores were deduced from the character and frequency of errors in a 20-score system.

In both collaborative tests carried out in parallel 16 panels participated each consisting of 5 members except one which had 6 members.

Each of the panels were given six coded samples, that is 3 samples in parallel.

In the course of evaluating the results of collaborative test I we obtained four outliers by the Dixon test. Using the Bartlett test four panels were outliers with their judgement of "consistency" (by mouth-feel) while two other panels were outliers by their judgement of "odour".

Similar results were obtained in collaborative test II. When evaluating the results by Dixon test eleven outliers were found. Using the Bartlett test four panels became outliers for their judgement of "consistency" by touching, 2 for their judgement upon "cross-section", 1 for its judgement of "taste" and 6 for their judgement of "consistency" by mouth-feel. The results of these two collaborative tests are shown in Table 1.

Table 1

Repeatibility (r) *and comparability* (R) *of the evaluation by sensory scoring (I, II) of Bologna sausage*

Properties tudies	WF	*r*		*R*	
		I	II	I	II
Appearance	0.4	0.8	0.9	1.8	1.5
Consistency (by touching)	0.2	0.7	1.2	2.2	2.6
Cross-section	0.8	0.7	0.8	1.3	1.7
Smell	0.4	1.0	0.9	2.7	2.6
Taste	1.6	0.8	0.8	2.4	3.3
Consistency (by mouth-feel)	0.6	1.1	1.0	2.7	1.9
Weighted total score	—	1.6	1.6	4.5	5.7

WF: weighting factor

Table 2

Repeatability (r) *and comparability* (R) *of the sensory scoring test of Bologna sausage (III, IV)*

Properties studied	WF	r		R	
		III	IV	III	IV
Appearance	0.6	0.7	0.4	1.1	0.5
Cross-section	1.0	0.7	0.7	1.7	0.8
Smell	0.8	0.6	0.5	0.9	0.6
Taste	1.6	0.5	0.7	1.3	0.8
Weighted total score	—	1.3	1.4	3.1	—1.6

Number of participating panels: 6
Number of Bologna sausage samples: 6
WF: weighting factor

The results of these two collaborative studies have shown that — apart from the diverse standards of the panels and other factors — a too detailed evaluation method or specifications of evaluation (property descriptive method II) and their inaccuracies (weighting factor method I) are not suitable for routine evaluation by sensory scoring. Therefore, in the next step, the weighting factor method (I), providing higher comparability, was made more exact and more simple, then it was tested in two collaborative studies in rapid succession under optimum conditions. The IIIrd and IVth sensory collaborative studies of Bologna sausage differed from one another only inasmuch as in the meantime the accuracy of the definition of concepts was increased, their unambiguousness improved and panel members acquired a greater experience in evaluating. This was shown by the diminishing of the number of highly diverging results. Within the frame of these two collaborative studies outliers were not found therefore the original results were used in evaluation. The results of these two collaborative studies are shown in Table 2.

Reproducibility as calculated from the data of both collaborative studies and related to the weighted total score is a highly favourable value.

On the whole the comparability of collaborative study IV may also be considered optimal. However, evaluation of the consistency within "cross-section" and "taste" caused still difficulties to the participating experts. This became apparent primarily in the reproducibility values. It may be considered as one of the conclusions of the collaborative studies that specifications of evaluation became more exact and became more suitable to routine application.

2.2. Sensory collaborative study of lager beer

The method laid down in HUNGARIAN STANDARD (1982a) served as a basis to the collaborative study of lager beer.

Table 3

Repeatability (r) *and comparability* (R) *of the sensory scoring test of lager beer*

Properties studied	WF	*r*	*R*
Stability of foam	0.5	1.2	1.7
Turbidity	0.5	1.1	1.7
Smell	0.6	1.0	1.6
Taste	0.6	1.0	1.5
Fullness of taste	0.6	1.1	1.7
Recency	0.6	1.2	1.4
Bitterness	0.6	1.0	1.5
Weighted total score	—	1.6	2.4

In relation to the interlaboratory collaborative study the instrumental measurement of turbidity as specified in the standard raised problems. Some of the participating panels gave up trying to carry it out. Since the guide to the collaborative study gave specifications of visual scoring as well, the results of the latter were used in evaluating.

The number of participating panels was: 19.

Number of the lager beer samples was: 4.

In evaluating the results of the collaborative study the Dixon, the Cochran and the Bartlett tests were used. Comparatively few outliers were found in the course of evaluation (6 by the Dixon test and 2 by the Cochran test) and these were replaced by the mean value of the remaining results.

There was no need to omit either panels or samples.

Repeatability and comparability as established in the collaborative study are shown in Table 3.

The r and R values obtained for the individual properties show that the sensory scoring of beers is not sufficiently unified. There is particularly great difference between results obtained by the same panel on the properties of "recency" and "stability of foam" when re-tested. Very high values were obtained for the reproducibility and comparability of the other properties, too.

Reproducibility (1.6) related to total score is somewhat high. The value for comparability (2.4) is acceptable. The values related to total score were advantageously influenced by the fact that the sensory evaluation of beer is based on more properties than the average and therefore the value of the weighting factors was in every case below 1.0.

2.3. Sensory collaborative study of cigarettes

The standard method previously widely discussed and tested (HUNGARIAN STANDARD, 1982b) was used in the collaborative study of cigarettes. In order

Table 4

Repeatability (r) *and comparability* (R) *of the sensory scoring of cigarettes*

Properties scored	WF	r	R
Appearance	0.4	0.5	1.2
Smell of product and of the main smoke	0.8	0.7	1.1
Taste, pungency of main smoke	1.4	0.6	1.3
Reaction to stimulus of main smoke	1.4	0.6	1.5
Weighted total score	—	1.3	2.8

to avoid the systematic error and reduce random error of sampling the samples for the collaborative study were prepared specially by one of the tobacco factories. The appearance of the samples (paper, filter, wrapping) was identical but they were filled with four different kinds of tobacco.

Number of panels participating was: 12,

number of cigarette samples was: 4.

The results of these collaborative studies were described in detail in another paper (MOLNÁR et al., 1985). It was stated that out of the 384 data only 11 were outliers and these were replaced by the averages of the remaining data. The results are given in Table 4.

The results have shown the developed collaborative system to be fundamentally suitable and the panels could reliably evaluate. The value of comparability should be, however, reduced by increasing the accuracy of evaluating, "reaction to stimulus, intensity of main smoke".

2.4. Sensory collaborative study of confectionary products

In this intralaboratory sensory study it became possible to study collectively several groups of products since the preconditions were given in the form of concurrent properties, defined satisfactorily in accord with the character of the product as well as of the uniformly built scoring test methods. Thus the collaborative study covered desserts, sweets, biscuits and hollow chocolate figures. The difference between the age of identical products was 3 to 12 months and this was perceptible in the quality of a part of the products.

Number of participating panels was 4, while that of the samples was 8.

The number of data-pairs reached 32 in the case of every property because the need to eject anyone of the panels did not arise. Outliers were found only in relation to reproducibility. The 7 outliers obtained by the Cochran test (the total number of data was 320) were replaced by the average value of the current remaining scores. Reproducibility and comparability of the weighted total score was calculated by average weighting factors.

Results are listed in Table 5.

Table 5

Repeatability (r) *and comparability* (R) *of the evaluation by sensory scoring of the confectionary products*

Property scored	Average WF	r	R
Shape	0.5	1.0	1.1
Colour of surface	0.6	0.7	0.8
Consistency	1.1	0.7	0.8
Smell	0.6	0.7	0.8
Taste	1.2	0.8	1.1
Weighted total score	—	1.5	1.8

Within the results favourable in their entity it can be established that the reproducibility of all the evaluations by scoring of the properties approximates the values of comparability, showing simultaneously the possibility of increasing the fidelity to judgement of the panels. Apart from "taste" reproducibility and comparability values for all the properties are almost identical which is conceivable only in intralaboratory studies. Comparability related to weighted total score compared to the results of other collaborative studies is the best but one. A value as low as this cannot be expected in interlaboratory sensory studies unless the laboratories are exceptionally well coordinated.

3. Conclusions

On the basis of the sensory collaborative studies carried out, the following can be concluded:

— The collaborative methodology is unambiguously suitable for the evaluation of data obtained by the signed scoring method of products included in the testing. Since differences of methodical character were not observed in the course of the sensory collaborative study of various kinds of food products, presumably the collaborative methodics developed can be used with every kind of food product for sensory evaluation by a sensory scoring method so designed.

— Certain conditions fulfilled, several similar product groups can be jointly exposed to sensory collaborative study leading to appropriate results.

The r and R values as determined by collaborative study are equivalent to or better than some comparable results determined abroad and published in related literature (NEUMANN et al., 1972; KOCHAN, 1976). These results are summed up in Table 6.

Table 6

Repeatability (r) *and comparability* (R) *of the weighted score in the sensory evaluation of different food products*

Product	*r*	*R*
Soft drinks I	2.0	2.7
Soft drinks II	1.8	2.5
Bologna sausage III	1.3	3.1
Bologna sausage IV	1.4	1.6
Lager beer	1.6	2.4
Cigarettes	1.3	2.8
Confectionary	1.5	1.8
Extra butter[a]	1.6	2.4
Powdered potato[a]	1.2	1.7
Liver sausage[a]	1.8	3.1
Roasted coffee[a]	1.8	3.0
Peppery sausage[b]	1.5	2.2

[a] Sensory collaborative study carried out by the author in GDR (NEUMANN et al., 1972)
[b] Results obtained by KOCHAN (1976)

The *r* and *R* values characterize the random error of the scoring methods which should be taken into consideration in classifying based on scores in the frame of quality control.

Sensory collaborative studies play an important role in the testing of evaluation specifications, description of methods, in their cross checking and increasing their accuracy as well as in coordination of the work of panels, in unification of the evaluation standards and in propagation of the correct carrying out of sensory evaluation. This was particularly proven in the results of collaborative studies of the evaluation of soft drinks and Bologna sausage, several times repeated.

Further experiences may permit to standardize evaluation specifications only after their collaborative study carried out under controlled conditions in which reproducibility does not exceed 1.6 and comparability 2.4. This requirement was fulfilled in 4 collaborative studies out of the total of 12 and only 2 of them were interlaboratory tests. This shows also that to fulfill the requirement on reproducibility and comparability well coordinated and reliable panels and unambiguous and exact methodological descriptions are needed.

The intralaboratory sensory collaborative studies (Bologna sausage III and IV and confectionary products) led to much more favourable values of reproducibility and comparability than interlaboratory studies did.

However, the results of interlaboratory collaborative study are generally not acceptable as the reproducibility and comparability values of sensory evaluation methods because the panels carrying out, in the frame of quality control, occasionally product control in parallel do not work at the same place. On the other hand, from the point-of-view of methodology their results are

extremely valuable because they mark the limits of scoring methods in relation to individual products.

The sensory collaborative study program appearing suitable on the basis of the results of collaborative study may become an organic part of sensory evaluation practice. However, because of the high costs sensory collaborative study can be applied only expediently and well planned.

New evaluation specifications developed for a definit group of products can be collaboratively studied on a certain prominent product. To supervise the work of panels, to compare the evaluation standard and to analyse the results the collaborative study, according to experiences up to the present, can also be utilized. In addition to the calculation of the results of the collaborative study data obtained are very useful in the determination of fidelity to judgement of panels and panel members and in carrying out other analyses, too (SZABÓ et al., 1987).

The experiences gained in sensory collaborative studies carried out so far have shown the collaborative methodology developed to meet the requirements of quality control taks and to be an important means of updating the sensory evaluation of foods.

Literature

HUNGARIAN STANDARD (1982a): *Sör. Érzékszervi bírálat.* (Beer. Sensory analysis.) MSZ 8761/4/82.

HUNGARIAN STANDARD (1982b): *Cigaretta érzékszervi vizsgálata.* (Sensory analysis of cigarettes.) MSZ 20568/82.

HUNGARIAN STANDARD (1984): *Vörösáruk. Párizsi.* (Frankfurters. Bologna.) MSZ 08-964/1/84.

KOCHAN, A. (1976): Problemenlösungen zur Qualitätssicherung in der Lebensmittelindustrie und Nahrungsgüterwirtschaft. Dissertation, Technische Universität, Dresden.

MOLNÁR, P., GÖRI, S. & KATONA, L. (1985): Cigaretta érzékszervi körvizsgálata. (Sensory collaborative study of cigarettes.) *Dohányipar, 1,* 31–33.

MOLNÁR, P. (1989): Adaptation of collaborative methodology to the sensory evaluation of foods by scoring. *Acta Alimentaria, 18,* 269—282.

NEUMANN, R., MOLNÁR, P. & BEER, E. (1972): Zur Standardisierung der sensorischen Butterprüfung und -bewertung. *Milchforschung—Milchpraxis, 14,* 33–37.

SZABÓ, E., MOLNÁR, P., GÓLYA, I. & ÁCS, P. (1967): Érzékszervi körvizsgálat eredményei üdítőitaloknál. (Results by sensory collaborative study on soft drinks.) *Élelmiszervizsg. Közl., 33,* 137–146.

BOOK REVIEW

International food regulation handbook

Policy — Science — Law

(Food Science and Technology Series No. 34.)

R. D. MIDDLEKAUF & P. SHUBIK (Eds)

Marcel Dekker, Inc. New York and Basel, 1989; 562 pages

This book is to present the current complex state of national and international approaches to the control of potential toxicological problems in the food supply. Although during the past several decades notable efforts have been directed toward centralizing approaches to these problems, much still remains to be done.

This book is intended to assist those for whom the regulatory differences are significant. Part I introduces the topics to be covered, including food, food ingredients, pesticides, microbiological and other contaminants, and nutrients. It also discusses the public policy considerations: their origins, their development within countries, and their expression in supranational organizations. In addition, this part reviews the role of nongovernmental bodies such as public interest and professional groups, and the various scientific considerations involved in evaluating the safety of foods and beverages.

Part II begins with a description of the general principles applied in regulating foods and beverages. Then the regulatory reguirements of selected countries are summarized according to a common outline to facilitate comparison of the applicable requirements of each jurisdiction and to provide a reference to where additional information may be obtained. The description of each country's regulations provides general guidance rather than specific details regarding any one ingredient. The conclusion of Part II identifies the trends in regulation that may be anticipated.

This volume will provide a guidance to the appropriate regulatory authority of concern under specific circumstances and for all the specialists who are dealing with the problems of food regulation and quality control.

I. VARSÁNYI

ANNOUNCEMENT

8TH WORLD CONGRESS OF FOOD SCIENCE AND TECHNOLOGY

September 29—October 4, 1991
Westin Harbor Castle Hotel
Toronto, Canada

Sponsored by the Canadian Institute of Food
Science and Technology
under the auspices of the International Union of Food
Science and Technology

For further information, please contact:

8th World Congress of Food Science and Technology
3340 Orlando Drive
Missisauga, Ontario
Canada 14V 1C7
Telephone: (416) 678-1220
Telex: 06968723
FYX: (416) 678-1229

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

RECENTLY ACCEPTED PAPERS

Investigation of chilling sensitivity of fruits and vegetables using Arrhenius plots
MURATA, T.

Mineral element content of edible and poisonous macrofungi
VETTER, J.

Mineral components and micro-elements in Jerusalem artichoke tubers grown in Hungary
BARTA, J., FODOR, P., TÖRÖK, SZ. & VUKOV, K.

Enzymatic and total amino acid changes under different storage conditions for damaged and undamaged beets
SPETTOLI, P., CURIONI, A., CRAPISI, A., VACCARI, G. & MANTOVANI, G.

Influence of boron on quality attributes of tomato fruit
ANKUSH, J. A., HARGITAI, L., BIACS, P. A. & DAOOD, H. G.

NOTICE TO CONTRIBUTORS

General. Manuscripts in English or Hungarian should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

Title. The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

Summary. The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

Text. The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

Symbols. Units, symbols and abbreviations should be used according to SI (System International) rules. Unusual abbreviations must be explained in the text or in an appendix.

Figures and tables. Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

References. The reference list (Literature) should be in alphabetical order as follows:

Periodicals: Names and initials of all the authors; year of publication in parentheses; colon; title of the paper; title of the periodical; inclusive page numbers.

Books: Names and initials of all the authors; the year of publication in parentheses; colon; title of the book; publishing firm, place of publication; inclusive page numbers.

Detailed instructions for publishing in Acta Alimentaria are available from the Editorial Office.

Authors will receive one set of proofs which must be corrected and returned at the earliest convenience to the Editorial Office. In this phase major alterations of the text cannot be accepted.

Offprints. We supply 100 offprints free of charge. Additional copies can be ordered.

No page charges are levied on authors or their institutions.

Acta Alimentaria is surveyed by Current Contents/Agriculture, Biology and Environmental Sciences, ASCA, BIOSIS, Nutrition Abstracts and Reviews.

ACTA ALIMENTARIA

VOLUME 18 No. 4 — 1989

CONTENTS

Index: 26.039